THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE BIOLOGICAL BULLETIN

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THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:00 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time

and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 9:00 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years. After having served two terms of four years each, Trustees are ineligible for re-election until a year has elapsed. In addition, there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. REPORT OF THE TREASURER

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen:

In the Treasurer's Report for 1950 it was stated that because of increasing costs "the margin is much too slim" and "additional income for current operating expenses is still of the utmost urgency." During 1951 this additional income was not forthcoming, the "margin" disappeared completely, and current expenses for the year exceeded current income by approximately $10,000. Total cash receipts, including $50,000 given by the Rockefeller Foundation for special apparatus, in addition to the $20,000 given for current expenses, were $362,293.29. Total expenditures, including $44,596.54 for this special apparatus, amounted to $371,531.82. As a result, cash on hand at the end of the year was only $3,853.96.

The results of the operations of the Mess and the Supply Department were especially disappointing. The Mess operated at a loss of $6,179.43. If depreciation and overhead charges are also deducted, the loss was $10,260.68. The Supply Department, after adjustments for the value of materials furnished to instruction and research, yielded a profit of $332.79. If depreciation is deducted, however, this is changed to a net loss of $3,189.36.

Because of higher prices for common stocks, the market value of the securities in the Endowment Funds increased by $39,862.37 during the year, to a total of $1,108,645.75. The average yield on securities was 4.63% on book value, 4.05% on market value. The investments and percentages in the Endowment Funds as of January 1, 1952 were:

	Book Value	%	Market Value	%
Bonds				
U. S. Government	$369,997.66	38.15%	$ 357,532.00	32.25%
Railroad	54,613.02	5.63	54,017.00	4.87
Public Utility	90,192.50	9.30	84,400.00	7.61
Industrial	34,800.00	3.59	33,110.00	2.99
Total Bonds	549,603.18	56.67	529,059.00	47.72
Preferred Stocks	127,173.50	13.11	115,627.00	10.43
Common Stocks	290,361.93	29.94	461,188.00	41.60
Total Securities	967,138.61		1,105,874.00	
Principal Cash	2,771.75	.28	2,771.75	.25
Totals	$969,910.36	100.00%	$1,108,645.75	100.00%

The Balance Sheet, Statement of Current Surplus, and Summary of Cash Transactions, as given in the report of the auditors, Seamans, Stetson and Tuttle of Boston, follow:

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DEC. 31, 1951

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of the Hanover Bank, New York, Trustee		$ 969,910.36	
Securities and Cash in Minor Funds		19,255.94	$ 989,166.30
Plant Assets:			
Land		$ 113,626.38	
Buildings		1,490,666.08	
Equipment		304,170.63	
Library		418,796.42	
		$2,327,259.51	
Less Reserve for Depreciation		817,862.69	$1,509,396.82
Current Assets:			
Cash		$ 13,340.77	
Mortgage Note Receivable		2,350.00	
Accounts Receivable		18,637.27	
Inventories:			
Supply Department	$ 54,719.00		
"Biological Bulletin"	16,467.20	$ 71,186.20	
Investments:			
Devil's Lane Property	35,347.55		
Stock in General Biological Supply House	12,700.00		
Other Investment Securities	46,101.25		
Retirement Fund	21,740.46	115,889.26	
Prepaid Insurance		6,406.32	
Items in Suspense (Debits)		1,716.87	$ 229,526.69
			$2,728,089.81

Liabilities

Endowment Funds:			
Endowment Funds	$ 967,821.66		
Reserve for Amortization	2,088.70	$ 969,910.36	
Minor Funds		19,255.94	$ 989,166.30
Plant Funds:			
Mortgage Notes Payable		$ 11,000.00	
Donations and Gifts	$1,375,719.68		
Other Investments in Plant from Gifts and Current Funds	122,677.14	1,498,396.82	$1,509,396.82
Current Liabilities and Surplus:			
Accounts Payable		$ 11,319.23	
Items in Suspense (Credits)		1,055.00	
Current Surplus		217,152.46	$ 229,526.69
			$2,728,089.81

Current Surplus Account

Balance, January 1, 1951			$ 231,514.60
Add:			
Excess of Income over Expense for Year		$ 14,245.09	
Reserve for Depreciation, 1951, Charged to Plant Funds		32,181.00	46,426.09
			$ 277,940.69
Deduct:			
Payments during year for Plant Assets:			
Equipment:			
From Current Funds	$ 4,344.66		
From Rockefeller Foundation Grant	44,020.64	$ 48,365.30	
Library:		12,422.93	60,788.23
Balance, December 31, 1951			$ 217,152.46

Summary of Cash Transactions for Year ended December 31, 1951

		Expenditures		
Current Cash	Receipts	Current	Additions to Capital Assets	Total Expenditures
Income from Endowment Funds	$ 44,898.43			
Income from Other Investments	22,517.60			
Donations, Rockefeller Foundation	70,000.00			
Donations, Others	5,240.00			
Real Estate Rentals	7,999.92	$ 1,011.75		$ 1,011.75
Membership Dues	2,442.00			
Instruction	15,170.00	10,229.11		10,229.11
Research (including Apparatus and Chemical Departments)	28,955.00	29,748.17	$ 44,596.54	74,344.71
Supply Department	68,883.66	77,563.91		77,563.91
Mess	45,843.27	54,694.47	1,285.48	55,979.95
Dormitories	22,405.56	7,970.10		7,970.10
"Biological Bulletin"	8,432.28	11,676.82		11,676.82
Library	4,539.26	9,593.12	9,161.99	18,755.11
Dr. Frank R. Lillie Memorial Fund—Arbacia Survey	3,070.03	2,616.28		2,616.28
Miscellaneous (Net)	1,437.74			
Office of Naval Research	1,998.61	2,000.00		2,000.00
Book Purchase Account, Woods Hole Oceanographic Institution	1,200.00	1,100.27		1,100.27
Real Estate Transactions	7,259.93	7,350.00		7,350.00
Administration		26,448.66		26,448.66
Buildings and Grounds		52,368.61	2,483.28	54,851.89
Interest, Insurance and Other Property Maintenance		9,954.46		9,954.46
Payments to Retirement Fund		9,678.80		9,678.80
	$362,293.29	$314,004.53	$ 57,527.29	$371,531.82

Cash Balance, January 1, 1951:		
The Hanover Bank	$ 11,003.27	
Falmouth National Bank	2,089.22	$ 13,092.49
Receipts		362,293.29
		$375,385.78
Payments		371,531.82
Cash Balance, December 31, 1951:		
The Hanover Bank	$ 2,923.71	
Falmouth National Bank	930.25	$ 3,853.96

Other Cash Accounts	Balances Jan. 1, 1951	Received	Paid	Balances Dec. 31, 1951
Allen R. Memhard Fund Cash	$ 83.05	$ 21.10		$ 104.15
Rev. Arsenious Boyer Burse Cash	182.07	116.75		298.82
Lucretia Crocker Fund Cash	674.92	424.91	$ 360.00	739.83
G. H. A. Clowes Fund Cash	1,000.00			1,000.00
Dr. Frank R. Lillie Memorial Fund Cash	8,331.84	2,725.00	3,070.03	7,986.81
Retirement Fund Cash	999.35	10,131.10	8,635.00	2,495.45
Bio Club Scholarship Fund Cash	72.53	54.71		127.24
Book Fund Cash	3,293.48		3,293.48	
Lalor Foundation Fellowships Cash	2,060.93	6,000.00	6,706.51	1,354.42

Respectfully submitted,
DONALD M. BRODIE,
Treasurer

V. REPORT OF THE LIBRARIAN

The appropriation for 1951 Library expenses was $14,255.68, plus $4500 from the Woods Hole Oceanographic Institution to be applied to staff salaries. This sum was expended as follows: Serials, $5472.90; Books, $424.50; Back Sets, $698.40; Binding, $2565.68; Supplies and Sundries, $443.19; Freight and Express, $105.13; Insurance, $45.00; Salaries, $9046.00. The total expenditures totalled $18,800.80.

In addition to the above, $1200 (plus a balance of $59.10 from 1950) was received from the Woods Hole Oceanographic Institution for acquisitions covering the field of oceanography. Of this amount, $1127.02 was spent, leaving a balance of $132.08 on Dec. 31, 1951.

The number of current journals received in 1951 remains the same as that for 1950, the new titles offsetting those that have ceased publication. There were 454 (11 new) Marine Biological Laboratory subscriptions; 587 (22 new) exchanges and 161 (14 new) gifts; 72 (7 new) were Woods Hole Oceanographic Institution subscriptions; 173 (13 new) were exchanges and 30 (6 new) were gifts; making a total of 1477 (73 new) current periodicals.

The Marine Biological Laboratory purchased 53 books, received 12 complimentary copies from authors, 45 titles from publishers, and 13 miscellaneous donations. The Woods Hole Oceanographic Institution purchased 26 titles, and received 10 gifts. The total number of new books placed on the shelves was 159.

During 1951, the balance of the Carnegie Corporation of New York Fund was expended. The final report, covering the years 1942–1951, shows that the $25,000 was spent for the following: 92 completed back sets; 111 partially completed back sets; 544 volumes bound; and 52 books (classics). This grant has enabled the Library to lower the number of sets needed. In addition, 29 sets were made nearer complete, most of these being war volumes which had been difficult to secure. The Woods Hole Oceanographic Institution acquired 9 back sets.

The reprint collection was increased by 4714 papers, 1604 being of current issue and the remaining ones of earlier date.

Forty-five volumes were borrowed on inter-library loan and 86 were sent to out-of-town institutions. Seventy-eight microfilm orders were filled, amounting to $141.82.

The amount of $1207.84 was received from the sale of accumulated duplicate journal sets and reprints. A portion of this sum was used very advantageously towards the purchase of a complete set of the Berlin Academy's valuable publications.

The Library had the great fortune to receive the reprint collection belonging to the late Dr. Otto Glaser. In sorting out these papers, many valuable ones were found not to appear in the Library. The bequest on the part of Dr. Glaser symbolized his ever-ready willingness to be of assistance to the Staff and his great interest in the growth of the Library.

Grateful acknowledgment is made to Dr. Roberts Rugh and to Dr. Mary E. Collett for their gifts. Several valuable books were used to replace badly worn copies. The gifts of reprints, including Dr. Glaser's, totalled 11,437, of which 1550 were not already in the collection.

At the end of the year 1951, the Library contained 61,068 bound volumes and 178,795 reprints.

The Librarian wishes to express her appreciation for the willing help and valuable advice received from the Library Committee throughout the year.

Respectfully submitted,
DEBORAH L. HARLOW,
Librarian

VI. REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen:

I submit herewith a report of the sixty-fourth session of the Marine Biological Laboratory for the year 1951.

During the past year important progress has been made in certain directions including scientific instrumentation, plant improvements, fellowship programs, course developments and collecting facilities. However, the Laboratory will be faced in the near future by serious financial problems which can be adequately met only with carefully planned aggressive action.

1. Scientific Equipment

The research possibilities of the Laboratory have been greatly enhanced by the acquisition of important and costly research equipment through a grant generously provided by the Rockefeller Foundation. Mr. Chester I. Barnard, then President of the Rockefeller Foundation, and Dr. Warren Weaver, Director of the Natural Sciences Division, became fully cognizant of the apparatus needs of the Laboratory as a result of their visit during the summer of 1950. The Laboratory applied for and received a grant of $75,000 which has made possible the acquisition of an ultra-centrifuge, preparation centrifuge, freeze-drying apparatus, refrigerated centrifuge, Warburg microrespirometers, spectrophotometers, ice-making machine, electronics apparatus, emergency generator and other equipment. The acquisition of this equipment is fully justified by its almost continuous use in research and teaching. As of next year there will be added to our equipment an electron microscope acquired under the same grant.

2. Plant Improvements

Continued progress was made during the past year in putting our plant in first class condition. Extensive exterior repairs were required by some of the wooden dormitory buildings. When these have been completed, work will then be pushed on the interiors which require considerable repair.

3. Instruction

The course in Marine Ecology will be inaugurated in the summer of 1952 under the direction of Dr. Bostwick H. Ketchum. The establishment of this course has been made possible through a grant of $4000 from the New York Zoological Society. It is hoped that this course will help to broaden the base of research interest at the Laboratory.

Dr. Daniel Mazia was appointed to succeed Dr. E. S. Guzman Barron in charge of the course in Physiology.

4. Fellowships

In addition to the Lalor Fellowships which have been so successful over the past several years, there is now available a new fellowship in Neurophysiology, established by the Grass Trust. There were two Grass Fellows this past season. The excellent results achieved in this first year are most promising for a continuation of this fellowship.

Through the generosity of Mrs. Frank R. Lillie, there has been established the Frank R. Lillie Educational Fund which will support a fellowship and provide research space at the Laboratory, starting in the summer of 1952. The fellowship provisions permit the selection of a fellow in Embryology or the broad field of general zoology.

5. Contributions and Gifts

The contribution of the Associates of the Laboratory totaled $1920. It was initially planned with this fund to have our boat carpenters build a boat to replace the Sagitta. However, the cost of construction frames proved prohibitive. As the result of a careful survey, it was found that the Sagitta could be put in first class condition by replacing the ribs, certain of the planking, most of the hardware and

part of the deck. This has been achieved most successfully. Mrs. Murray Crane made a special contribution of $300 to help cover the costs of this remodeling. Mrs. Edward B. Meigs contributed $500 in memory of her husband, Dr. Edward Browning Meigs, who was a generous friend of the Laboratory and an active investigator.

Mr. and Mrs. George F. Jewett contributed $100 for general Laboratory purposes.

Dr. W. D. Curtis showed his continued interest in the Supply Department by the gift of an outboard motor to provide tender service for the Dolphin on class trips.

These added special gifts from these Associates are all most gratefully acknowledged.

The Kettering Foundation contributed $2500 to the Lillie Memorial Fund. This fund has proved invaluable for the development of special marine collecting facilities. During the past few years there has been a striking interest in a diversity of animal and botanical forms which has required the development of special collecting and transporting methods.

6. Government Contracts

The Laboratory has a contract with the Office of Naval Research which provides funds and facilities for the collecting and transport of squid from distant points to the Laboratory. The methods that have been developed have proved most effective and require refrigerated, aerated transport tanks.

A contract is being negotiated with the Atomic Energy Commission to obtain support of the radioisotopes research. It is hoped that research assistants, apparatus and isotopes may be obtained through such a contract.

7. Bar Neck Property

There was some prospect that the Navy might acquire the Bar Neck Property for a Navy Oceanographic building. There was strong opposition to this from the Trustees of the Marine Biological Laboratory who were strongly opposed to parting with this very important Laboratory asset, since it did not appear that this site was essential for the proper operation of the projected laboratory. No final solution of this problem has been worked out but it is probable that the appropriation for the land and building is hardly sufficient to permit the acquisition of such a valuable piece of property as the building site.

8. Grants

The Laboratory still continues to receive support from the Rockefeller Foundation on a grant made three years ago. This grant has two more years to run with $35,000 available for these two years. This grant has been used for the general running expenses of the Laboratory. Its termination will result in a serious reduction in the funds for these general expenses. Plans must be developed this year to offset this loss in income resulting from the termination of this grant. In fact, it is essential that even more than this be done, that additional funds be found over and above those which have been so generously provided by the Rockefeller Foundation. The wage level for the full-time staff of the Laboratory has not kept pace with the increased cost of living. Also, with an increased income, many improvements can be made in the services rendered to investigators, increasing the effective-

ness of their work. The housing situation is also susceptible of considerable improvement.

These problems should receive careful consideration by the Trustees and plans developed which will show real promise of solving the difficulties.

Respectfully submitted,
PHILLIP B. ARMSTRONG,
Director

1. MEMORIAL

Walter Eugene Garrey

By

F. P. KNOWLTON

Walter Eugene Garrey was born in Reedsville, Wisconsin, April 7, 1873, the elder son of a pioneer Wisconsin physician, John Eugene and Harriet Anderson Garrey. After receiving his elementary education in the public schools of Wisconsin, he entered Lawrence University from which he was graduated with the degree of Bachelor of Science in 1894. From 1894 to 1898 he was an extension instructor in the schools of Chicago. The year 1898 was spent as a student at the University of Berlin, following which he was appointed Fellow in Physiology in the department of Professor Jacques Loeb at the University of Chicago. There, in 1900, he received the degree of Doctor of Physiology and Pharmacology at Cooper Medical College at San Francisco. During these years at San Francisco he came east each summer to study medicine at Chicago, finally receiving the M.D. degree from Rush Medical College in 1909.

From 1910 to 1916 he was Associate Professor of Physiology in Washington University at St. Louis. In 1916 he was appointed Professor of Physiology at Tulane University where he remained until appointed to the chair of Physiology in the reorganized medical school of Vanderbilt University at Nashville. He served as Professor of Physiology and Chairman of the department at Vanderbilt from 1925 until 1944 when he was appointed Emeritus Professor.

The first course in Physiology at the Marine Biological Laboratory was given by Professor Jacques Loeb in 1899. As Fellow in Physiology, Garrey assisted in the collection and preparation of material for the course. From that time on, excepting only the years he was in San Francisco, his summers regularly were spent at the Woods Hole laboratory. He was an instructor in the course in Physiology until 1925, and continued as research worker and consultant in Physiology until the year of his death. In 1920 the Corporation elected him a member of the Board of Trustees. He served as trustee until 1944 when he became Emeritus Trustee.

Of some 60 papers on which his name appears as author or co-author, nearly half relate to experiments carried out, in whole or in part, in this Laboratory. A fundamental interest in general physiology resulting from his early association with Professor Loeb appears in many of these papers. The physiology of the heart was his major interest throughout most of his life. His experiments on cardiac fibrillation, and a theory of fibrillation based on these experiments, will be remembered as an outstanding contribution to medical science.

Membership on the National Research Council, the National Board of Medical Examiners, and the Council on Physical Therapy of the American Medical Association give evidence of the high esteem in which he was held among scientific workers in diverse fields. The highest possible tribute from colleagues in his own special field came when he was chosen president of the American Physiological Society in 1937 and 1938.

An athlete in his younger days, he remained tall and erect. With his head of white hair he was a noteworthy figure in any gathering. A somewhat gruff exterior covered a friendly and sympathetic personality. As Consultant in Physiology at the Laboratory, his advice and assistance were always freely available to younger workers. A number of papers from this Laboratory which do not bear his name nevertheless owe their origin to suggestions and advice from Dr. Garrey. He was fond of entertainment and of entertaining, and the fact that Mrs. Garrey was a charming hostess made their home a frequent gathering-point for friends and neighbors. Though illness handicapped him in his last years he retained his interest in scientific work; and only last autumn as he journeyed back to Nashville he was outlining work that he hoped to complete. However, further illness intervened and he died at Nashville, June 15, 1951.

The Corporation and members of the Laboratory record the passing of one of those individuals whose broad interests and noteworthy accomplishments have helped to make the reputation of the Laboratory international. Those of us who knew him mourn the loss of an honored friend.

2. The Staff, 1951

Philip B. Armstrong, Director, State University of New York, School of Medicine, Syracuse.

Senior Staff of Investigation

E. G. Conklin, Professor of Zoology, *Emeritus,* Princeton University.
R. S. Lillie, Professor of General Physiology, *Emeritus,* The University of Chicago.
A. P. Mathews, Professor of Biochemistry, *Emeritus,* University of Cincinnati.
G. H. Parker, Professor of Zoology, *Emeritus,* Harvard University.

ZOOLOGY

I. Consultants

F. A. Brown, Jr., Professor of Zoology, Northwestern University.
Libbie H. Hyman, American Museum of Natural History.
A. C. Redfield, Woods Hole Oceanographic Institution.

II. Instructors

L. H. Kleinholz, Associate Professor of Biology, Reed College, in charge of course.
George M. Moore, Professor of Zoology, University of New Hampshire.
C. G. Goodchild, Professor of Biology, S. W. Missouri State College.
John H. Lochhead, Associate Professor of Zoology, University of Vermont.
Madelene E. Pierce, Associate Professor of Zoology, Vassar College.
Ralph I. Smith, Assistant Professor of Zoology, University of California.
T. H. Waterman, Assistant Professor in Biology, Yale University.
Marion H. Pettibone, Arctic Research Laboratory, U. S. National Museum.

III. Laboratory Assistants

Robert S. Howard, Northwestern University.
Muriel Sandeen, Duke University.

EMBRYOLOGY

I. Instructors

S. Meryl Rose, Associate Professor of Zoology, University of Illinois, in charge of course.

CHARLES B. METZ, Assistant Professor of Zoology, Yale University.
JOHN T. BONNER, Assistant Professor of Biology, Princeton University.
MAC V. EDDS, JR., Assistant Professor of Biology, Brown University.
JOHN R. SHAVER, Assistant Professor of Zoology, University of Missouri.
EDGAR ZWILLING, Associate Professor of Genetics, University of Connecticut.

II. LABORATORY ASSISTANTS

J. BRUCE GUYSELMAN, Northwestern University.
ALBERT E. MURRAY, JR., University of Illinois.

PHYSIOLOGY

I. CONSULTANTS

MERKEL H. JACOBS, Professor of Physiology, University of Pennsylvania.
OTTO LOEWI, Professor of Pharmacology, New York University, School of Medicine.
ARTHUR K. PARPART, Professor of Biology, Princeton University.
ALBERT SZENT-GYORGYI, Director, Institute for Muscle Research.

II. INSTRUCTORS

E. S. GUZMAN BARRON, Associate Professor of Biochemistry, University of Chicago, in charge of course.
DANIEL MAZIA, Professor of Zoology, University of California.
IRVING M. KLOTZ, Professor of Chemistry, Northwestern University.
STEPHEN KUFFLER, Associate Professor, Johns Hopkins University.
H. BURR STEINBACH, Professor of Zoology, University of Minnesota.
GEORGE WALD, Professor of Biology, Harvard University.

III. LABORATORY ASSISTANT

ANDREW NEMETH, Johns Hopkins University School of Medicine.

BOTANY

I. CONSULTANTS

BOSTWICK H. KETCHUM, Woods Hole Oceanographic Institution.
WM. RANDOLPH TAYLOR, Professor of Botany, University of Michigan.
GEORGE F. PAPPENFUSS, Professor of Botany, University of Michigan.

II. INSTRUCTORS

MAXWELL S. DOTY, Assistant Professor of Biology, University of Hawaii, in charge of course.
R. D. WOOD, Assistant Professor of Botany, Rhode Island State College.
FRANCIS T. HAXO, Assistant Professor of Biology, Johns Hopkins University.

III. LABORATORY ASSISTANT

JULIUS SILBERGER, Harvard University.

IV. COLLECTOR

ALBERT J. BERNATOWICZ, University of Michigan.

V. Lecturers

J. B. Lackey, Medical and Scientific Editor, The Blakiston Company.
Ruth Patrick, Curator of Limnology, Academy of Natural Sciences of Philadelphia.
John Wallace, Academy of Natural Sciences of Philadelphia.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Mrs. Deborah Lawrence Harlow, Librarian
Mary A. Rohan — Loretta J. Benevides — Joan M. Hoffer

APPARATUS DEPARTMENT

Robert Mills, Manager
James D. Graham — Betty J. Keeler — Seaver R. Harlow

CHEMICAL DEPARTMENT

Gail M. Cavanaugh, Chemical Supplies Manager

SUPPLY DEPARTMENT

James McInnis, Manager
John S. Rankin, Naturalist

Ruth Crowell		Patricia M. Conway
M. B. Gray	G. Lehy	James Whitcomb
A. M. Hilton	Carl O. Schweidenback	H. S. Wagstaff
W. E. Kahler	R. O. Lehy	Robert Perry

GENERAL OFFICE

Homer P. Smith, General Manager
Polly L. Crowell, Office Manager
Mrs. Lila S. Myers — Mrs. Mary M. Carr

GENERAL MAINTENANCE

R. W. Kahler, Superintendent

Robert Adams	James S. Thayer
Richard Alberts	Albert Neal
Robert Gunning	Alton J. Pierce
John H. Head	T. E. Tawell

George A. Kahler

3. Investigators and Students

Independent Investigators, 1951

Allen, M. Jean, Assistant Professor of Zoology, University of New Hampshire.
Allfrey, Vincent, Assistant, The Rockefeller Institute for Medical Research.
Alscher, Ruth Paula, Assistant Professor of Biology, Manhattanville College of the Sacred Heart.

AMBERSON, WILLIAM R., Professor of Physiology, University of Maryland Medical School.
ANDERSON, RUBERT S., Professor of Physiology, University of South Dakota.
ARMSTRONG, PHILIP B., Professor of Anatomy, State University of New York at Syracuse.
BAILY, NORMAN A., Research Scientist, Columbia University.
BARRON, E. S. GUZMAN, Associate Professor of Biochemistry, University of Chicago.
BARTLETT, JAMES H., Professor of Physics, University of Illinois.
BARTON, JAY, II, Instructor in Zoology, Columbia University.
BENESCH, REINHOLD, Fellow in Experimental Medicine, Northwestern University.
BERGER, CHARLES A., Director, Biological Laboratory, Fordham University.
BEVELANDER, GERRIT, Professor of Histology, New York University.
BISHOP, DAVID W., Professor of Physiology, University of Massachusetts.
BLOCH, EDWARD H., Investigator, American Heart Association, Western Reserve University.
BLUM, HAROLD F., Physiologist, N.C.I., and Visiting Lecturer, Princeton University.
BOETTIGER, EDWARD P., Assistant Professor of Zoology, University of Connecticut.
BONNER, JOHN TYLER, Assistant Professor of Biology, Princeton University.
BOREI, HANS, Visiting Professor, University of Pennsylvania.
BRIDGMAN, JOSEPHINE, Agnes Scott College.
BRONK, DETLEV W., President, Johns Hopkins University.
BROOKS, MATILDA M., Research Associate, University of California.
BROWN, F. A., JR., Chairman, Dept. Biological Sciences, Northwestern University.
BRUNST, VICTOR V., Research Associate, University of Maryland Medical School.
BURBANCK, W. D., Professor of Biology, Emory University.
BUTLER, ELMER G., Professor of Zoology, Princeton University.
CHARLES, DONALD R., Chairman, Dept. of Biology, University of Rochester.
CHASE, AURIN M., Associate Professor of Biology, Princeton University.
CHENEY, RALPH HOLT, Professor of Biology, Brooklyn College.
CHITWOOD, B. G., Associate Professor of Biology, Catholic University.
CIERESZKO, LEON S., Assistant Professor of Chemistry, University of Oklahoma.
CLAFF, C. LLOYD, Research Associate in Surgery, Harvard Medical School.
CLARK, ARNOLD M., Associate Professor of Biology, University of Delaware.
CLARK, ELIOT R., Professor, *Emeritus*, of Anatomy, University of Pennsylvania.
CLEMENT, A. C., Associate Professor of Biology, Emory University.
CLOWES, G. H. A., Research Director, *Emeritus*, Eli Lilly and Company.
COHEN, ADOLPH I., Graduate Student, Columbia University.
COHEN, SEYMOUR S., Associate Professor Physiol. Chemistry and Pediatrics, Children's Hospital, Philadelphia.
COLWIN, ARTHUR L., Assistant Professor of Biology, Queens College.
COLWIN, LAURA N., Queens College.
CONKLIN, EDWIN G., Professor of Biology, *Emeritus*, Princeton University.
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COPLEY, ALFRED L., Assistant Clinical Professor of Medicine, New York Medical College.
CORNMAN, IVOR, Assistant Research Professor in Anatomy, George Washington University School of Medicine.
COSTELLO, DONALD P., Kenan Professor of Zoology, University of North Carolina.
CROWELL, SEARS, Assistant Professor of Zoology, Indiana University.
CURTIS, W. C., Professor of Zoology, *Emeritus*, University of Missouri.
DAN, KATSUMA, Professor of Biology, Tokyo Metropolitan University, Tokyo, Japan.
DIETER, C. D., Head of Department of Biology, Washington-Jefferson College.
DILLER, WILLIAM F., Assistant Professor of Zoology, University of Pennsylvania.
DOTY, MAXWELL S., Associate Professor of Botany, University of Hawaii.
DURYEE, WILLIAM R., Cytologist, Carnegie Institution.
EDDS, M. V., JR., Assistant Professor of Biology, Brown University.
ELLIOTT, ALFRED M., Professor of Zoology, University of Michigan.
FAILLA, G., Professor of Radiology, Columbia University.
FERNANDO, HENRY E., Instructor in Entomology, Dept. Agriculture, Ceylon.
FISHER, JOSEPH D., Research Biochemist, Armour Laboratories, Chicago.
FRIES, E. F. B., Assistant Professor, City College of New York.
GAFFRON, HANS, Associate Professor of Biochemistry, University of Chicago.

GEREN, BETTY BEN, Research Associate, Massachusetts Institute of Technology.
GILMAN, LAUREN C., Associate Professor of Zoology, University of Miami.
GOODALL, M. C., Research Fellow, Institute for Muscle Research.
GOODCHILD, CHAUNCEY G., Professor of Biology, S. W. Missouri State College.
GREEN, JAMES W., Assistant Professor of Physiology, Rutgers University.
GROSCH, DANIEL S., Associate Professor of Zoology, North Carolina State College.
GRUNDFEST, HARRY, Associate Professor of Neurology, College of Physicians and Surgeons.
GUTTMAN, RITA, Assistant Professor, Brooklyn College.
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HARVEY, ETHEL BROWNE, Independent Investigator, Princeton University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HAXO, FRANCIS T., Assistant Professor of Biology, Johns Hopkins University.
HEILBRUNN, L. V., Professor of Zoology, University of Pennsylvania.
HELLER, JOHN HERBERT, Chairman, Medical Physics Study Unit, Yale University School of Medicine.
HENDLEY, CHARLES D., Associate Professor of Pharmacology, University of South Dakota.
HENLEY, CATHERINE, Research Associate, University of North Carolina.
HODES, ROBERT, Professor of Neurophysiol. and Exp. Neurology, Tulane University School of Medicine.
HORVATH, BENI, Institute for Muscle Research.
JACOB, MIRIAM I., Graduate Student, New York University.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JACOBS, WILLIAM P., Assistant Professor of Biology, Princeton University.
JENKINS, GEORGE B., Professor of Anatomy, *Emeritus*, George Washington University.
JENSEN, AAGE BOHUS, Research Fellow, Institute for Cancer Research, Fox Chase, Philadelphia.
JØRGENSEN, C. BARKER, University Adjunct, University of Copenhagen.
KARNOVSKY, M. L., Associate, Harvard University Medical School.
KARUSH, FRED, Assistant Professor, Children's Hospital, Philadelphia.
KELLY, SALLY, M., Assistant Professor of Plant Science, Vassar College.
KEMPTON, RUDOLF T., Professor of Zoology, Vassar College.
KEOSIAN, JOHN, Professor of Biology, Rutgers University.
KIND, C. ALBERT, Assistant Professor of Chemistry, University of Connecticut.
KING, JOHN W., Professor of Biology, Morgan State College.
KISCH, BRUNO, Professor of Chemistry, Yeshiva University.
KLEINHOLZ, LEWIS H., Professor of Biology, Reed College.
KLOTZ, IRVING M., Professor of Chemistry and Biology, Northwestern University.
KRAHL, MAURICE E., Associate Professor of Biochemistry, Washington University School of Medicine.
KUFFLER, STEPHEN W., Associate Professor, John Hopkins University.
KUNTZ, ELOISE, Instructor in Physiology, Vassar College.
LANSING, ALBERT I., Associate Professor of Anatomy, Washington University.
LAVIN, GEORGE I., The Rockefeller Institute for Medical Research.
LAYTON, LAWRENCE L., Assistant Professor of Biochemistry, Johns Hopkins University.
LAZAROW, ARNOLD, Associate Professor of Anatomy, Western Reserve University.
LEFEVRE, PAUL G., Associate Professor of Physiology and Biophysics, University of Vermont.
LEVEQUE, THEODORE F., Research Assistant, Dept. Anatomy, University of Colorado School of Medicine.
LEVY, MILTON, Associate Professor of Chemistry, New York University.
LIBET, BENJAMIN, Assistant Professor of Physiology, University of California Medical School.
LILLIE, RALPH S., Professor of Physiology, *Emeritus*, University of Chicago.
LILIEN, OTTO M., Research Fellow, Jefferson Medical College.
LING, GILBERT N., Instructor in Physiol. Optics, Wilmer Institute, Johns Hopkins Hospital.
LOCHHEAD, JOHN H., Associate Professor of Zoology, University of Vermont.
LOESER, CHARLES N., Instructor in Anatomy, Western Reserve University.
LOVE, WARNER E., Research Fellow in Biophysics, Johnson Foundation, University of Pennsylvania.
LOVELACE, ROBERTA, Adjunct Professor of Biology, University of South Carolina.

LUCKÉ, BALDUIN, Professor of Pathology, University of Pennsylvania.
LYNCH, WILLIAM F., Professor of Biology, St. Ambrose College.
MARSLAND, DOUGLAS, Professor of Biology, New York University, Washington Square College.
MARTIUS, CARL, Professor, Physiol.-chèm. Institut, Tübingen, Germany.
MASON, HOWARD S., Research Associate, Princeton University.
MAXFIELD, MYLES, Research Associate, Massachusetts Institute of Technology.
MAZIA, DANIEL, Associate Professor of Zoology, University of California.
METZ, CHARLES B., Assistant Professor of Zoology, Yale University.
MIHALYI, ELEMER, Research Fellow, Institute for Muscle Research.
MITCHELL, RUTH, Research Associate, Tufts Medical School.
MOORE, GEORGE M., Professor of Zoology, University of New Hampshire.
MULLINS, L. J., Associate Professor, Purdue University.
MUSACCHIA, XAVIER J., Instructor, Biological Laboratories, St. Louis University.
NACHMANSOHN, DAVID, Assistant Professor of Neurology, College of Physicians and Surgeons.
NELSON, LEONARD, Instructor of Physiology, University of Nebraska.
NEJELSKI, LEO L., JR., Research Associate, Institute for Muscle Research.
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OSTERHOUT, W. J. V., Member, *Emeritus*, Rockefeller Institute for Medical Research.
PACKARD, CHARLES, Woods Hole, Massachusetts.
PARMENTER, CHARLES L., Professor of Zoology, University of Pennsylvania.
PARPART, ARTHUR K., Chairman, Department of Biology, Princeton University.
PASSANO, LEONARD M., III, Graduate Student, Yale University.
PEACOCK, SAMUEL M., Instructor of Neurophysiology, Tulane University School of Medicine.
PERKINS, JOHN F., Assistant Professor of Physiology, University of Chicago.
PETTIBONE, MARION H., Researcher on Annelids, U. S. National Museum.
PIERCE, MADELENE E., Professor of Zoology, Vassar College.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
PROVASOLI, LUIGI, Chairman of Biology Department, St. Francis College.
RAUCH, HAROLD, Instructor in Zoology, University of Massachusetts.
RIESER, PETER, Instructor in Physiology, Fordham University.
RICE, MARY E., Research Associate, Columbia University.
RONKIN, RAPHAEL R., Assistant Professor of Physiology, University of Delaware.
ROSE, S. MERYL, Associate Professor of Zoology, University of Illinois.
ROTH, JAY S., Assistant Professor of Biological Chemistry, Hahnemann Medical College.
RUGH, ROBERTS, Associate Professor in Radiology, Columbia University.
RUTSTEIN, DAVID D., Professor of Preventive Medicine, Harvard Medical School.
SAETREN, HANS, Visiting Investigator, The Rockefeller Institute for Medical Research.
SCHAEFFER, ASA A., Professor of Biology, Temple University.
SCHMITT, FRANCIS O., Head of Department of Biology, Massachusetts Institute of Technology.
SCHNEIDER, LILLIAN K., Research Assistant in Microbiology, Columbia University.
SCHOLANDER, P. F., Office of Naval Research.
SCHULMAN, MARTIN P., Research Associate, University of Pennsylvania.
SCOTT, ALLAN C., Professor of Biology, Colby College.
SCOTT, SISTER FLORENCE MARIE, Professor of Biology, Seton Hill College.
SCOTT, GEORGE T., Associate Professor of Zoology, Oberlin College.
SHANES, ABRAHAM M., Physiologist, National Institutes of Health.
SHAVER, JOHN R., Assistant Professor of Zoology, University of Missouri.
SICHEL, F., Professor of Physiology and Biophysics, University of Vermont College of Medicine.
SINGH, INDERJIT, Professor of Physiology, Medical College, Agso, India.
SMITH, RALPH I., Assistant Professor of Zoology, University of California.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
SPRATT, NELSON T., Associate Professor of Zoology, University of Minnesota.
STEINBACH, H. B., Professor of Zoology, University of Minnesota.
STERN, HERBERT, Assistant to the Institute, Rockefeller Institute for Medical Research.

STEWART, DOROTHY R., Associate Professor and Head of Biology Dept., Rockford College.
STOKEY, ALMA G., Professor of Plant Science, *Emeritus*, Mount Holyoke College.
STUNKARD, HORACE W., Professor of Biology, New York University.
SZENT-GYORGYI, ALBERT, Chief Investigator, Institute for Muscle Research.
SZENT-GYORGYI, ANDREW G., Research Fellow, Institute for Muscle Research.
TAYLOR, WM. RANDOLPH, Professor of Botany, University of Michigan.
TOBIAS, JULIAN M., Associate Professor of Physiology, University of Chicago.
TRAGER, WILLIAM, Associate Member, Rockefeller Institute.
TRINKAUS, J. P., Assistant Professor of Zoology, Yale University.
TRUANT, ALDO P., Assistant Professor of Pharmacology, Tufts Medical School.
VILLEE, CLAUDE A., Assistant Professor of Biological Chemistry, Boston Lying-In Hospital.
WALD, GEORGE, Professor of Biology, Harvard University.
WARNER, ROBERT C., Assistant Professor of Chemistry, New York University College of Medicine.
WICHTERMAN, RALPH, Professor of Biology, Temple University.
WIERCINSKI, FLOYD J., Assistant Professor of Physiology, Hahnemann Medical College.
WILBER, CHARLES G., Director, Biological Laboratories, St. Louis University.
WILSON, WALTER L., Instructor in Physiology and Biophysics, University of Vermont, College of Medicine.
WOOD, RICHARD D., Assistant Professor of Botany, Rhode Island State College.
WRIGHT, PAUL, Professor of Zoology, University of Michigan.
WRINCH, DOROTHY, Lecturer in Physics, Smith College.
ZWILLING, EDGAR, Associate Professor, University of Connecticut.

Beginning Investigators, 1951

ALLEN, MARGARET D., Graduate Student, University of Pennsylvania.
ALLEN, ROBERT DAY, Fellow, National Institutes of Health, University of Pennsylvania.
BECKER, HAL C., Electronics Engineer (Biophysics), Tulane University School of Medicine.
BERMAN, RUTH, Graduate Student, Columbia University.
BRUMMETT, ANNA R., Graduate Student, Bryn Mawr College.
CHAET, ALFRED B., Graduate Student, University of Pennsylvania.
CLARK, MARGUERITE RUTH, Graduate Fellow, St. Louis University.
DAVIS, EDGAR F., Research Assistant, Princeton University.
FISHER, HARRIET, Analytical Chemist, Armour Laboratories.
GOLDSTEIN, LESTER, Assistant Instructor of Zoology, University of Pennsylvania.
GROSS, PAUL RANDOLPH, Graduate Student and Assistant Instructor in Zoology, University of Pennsylvania.
HERR, EARL B., JR., Graduate Student, University of Delaware.
HOFFMAN, JOSEPH F., Graduate Student, Princeton University.
HONEGGER, CAROL M., Instructor, Temple University.
INOUÉ, SHINYA, Graduate Student, Princeton University.
JACOBSON, ALICE A., Graduate Student, University of Pennsylvania.
KATZ, MICHAEL, Assistant Instructor, University of Pennsylvania.
MITCHELL, CONSTANCE, Instructor of Biology, University of Delaware.
MONER, JOHN G., Graduate Student, Princeton University.
PEYSER, PINCUS, Graduate Student, Columbia University.
PROCTOR, NATHANIEL K., Student, University of Pennsylvania.
RAY, DAVID T., Instructor in Zoology, Howard University.
RUDENBERG, F. HERMANN, Graduate Student, University of Chicago.
STROUT, PHYLLIS M., Student, Goucher College.
SULLIVAN, ROBERT LITTLE, Graduate Student, North Carolina State College.
THOMAS, LYELL J., Graduate Student, University of Pennsylvania.
TOWNSLEY, SIDNEY J., Graduate Student, Yale University.
WEBB, H. MARGUERITE, Assistant Professor of Biology, Boston College.

Research Assistants, 1951

ADELMAN, WILLIAM J., University of Vermont College of Medicine.
BALZER, DOROTHY T., Harvard Medical School.
BARBER, SAUL B., Osborn Zoological Laboratory, Yale University.
BENNETT, MICHAEL, Yale University.
BERNATOWICZ, ALBERT J., University of Michigan.
BRUMM, ANNE F., Harvard Medical School.
BULLOWA, ANNE, New York Medical College.
BURCHARD, JOHN E., Princeton University.
CHAPMAN, GEORGE B., National Cancer Institute.
CLENDENNING, K. A., National Research Council of Canada.
COHEN, MAX, Columbia University.
COWDRY, ALICE M., Wellesley College.
CROSS, HELEN, Columbia University.
CURRIER, JOANNE, Vassar College.
CURTISS, RUTH A., Indiana University.
DONOVAN, JOANNE E., Yale University.
DRUVA, EMILY, Columbia University.
FINGERMAN, MILTON, Northwestern University.
GAGNON, ANDRÉ, University of Pennsylvania.
GILLESPIE, RUTH J., Carnegie Institution.
GREENGARD, PAUL, Johns Hopkins University.
GUYSELMAN, J. BRUCE, Northwestern University.
HINES, MARGARET N., Northwestern University.
HODGE, ALAN J., Massachusetts Institute of Technology.
HOLLAND, JANICE K., Brown University.
HOWARD, ROBERT S., Northwestern University.
IAMPIETRO, P. F., University of Massachusetts.
JACOBSON, MARCUS A., White Plains, New York.
JOHNSON, PHYLLIS E., University of Chicago.
KAUZMANN, MRS. ELIZABETH, National Cancer Institute.
KAYE, ALVIN M., Columbia University.
KENT, DONALD E., University of North Carolina.
LANDAU, JOSEPH V., New York University.
LEONARD, LAWRENCE M., Harvard University.
LING, CHIUN-TONG, Johns Hopkins University.
MARTIN, B. JEAN, University of Minnesota.
MCINTYRE, JANE H., Harvard Medical School.
MERZ, TIMOTHY, Johns Hopkins University.
MILSTEIN, SEYMOUR W., Hahnemann Medical College.
MURRAY, ALBERT E., University of Illinois.
NEMETH, ANDREW, Johns Hopkins University School of Medicine.
PADAWER, JACQUES, New York University.
PAULING, PETER J., Columbia University.
ROBINSON, ELIZABETH B., Amherst College.
RUSZEL, S. FRANCES, Rockefeller Institute for Medical Research.
SANDEEN, MURIEL I., Duke University.
SEKI, SADA LOUISE, University of Chicago.
SIGHTS, WARREN P., University of Chicago.
SILBERGER, JULIUS, JR., Harvard College.
SLATER, JOHN V., University of Michigan.
TIETZE, FRANK, Washington University.
WHITCOMB, JOHN, Amherst College.

Library Readers, 1951

AMBRUS, JULIAN L., Associate Professor, Philadelphia College of Pharmacy and Science.
BOTSFORD, E. FRANCES, Professor of Zoology, Connecticut College.
BRACKETT, STERLING, Manager of Technical Service, Lederle Laboratories.
BURWELL, E. LANGDON, Practicing Physician, Woods Hole.
DAVIS, BERNARD D., Surgeon, Tubercular Research Lab., Cornell University Medical College.
DEKORNFELD, THOMAS J., Chevy Chase, Maryland.
DELAMATER, EDWARD D., Research Professor, University of Pennsylvania.
DORFMAN, ALBERT, Assistant Professor of Pediatrics, University of Chicago Clinics.
DUBOIS, EUGENE F., Professor of Physiology, *Emeritus*, Cornell University Medical College.
EICHEL, BERTRAM, Assistant Research Specialist, Rutgers University.
EICHEL, HERBERT J., Research Fellow, Rutgers University.
GABRIEL, MORDECAI L., Assistant Professor of Biology, Brooklyn College.
GUREVICH, VLADIMIR, Assistant Visiting Physician, Bellevue Hospital.
KABAT, ELVIN A., Associate Professor of Bacteriology, College of Physicians and Surgeons.
KAUZMAN, WALTER, Assistant Professor of Chemistry, Princeton University.
KEEFFE, MARY M., Assistant Professor of Biology, College of St. Thomas.
KRAMER, MOLLIE P., Bibliographer, American Meteorological Society.
LACHANCE, JEAN PAUL, Research Assistant, Laval University.
LOEWDIN, PER-OLOV, Research Associate, Duke University.
LOEWI, OTTO, Research Professor of Pharmacology, New York University.
MCDONALD, SISTER ELIZABETH SETON, College of Mount St. Joseph-on-the-Ohio.
MARINELLI, LEONIDAS D., Senior Biophysicist, Argonne National Laboratory.
MEMHARD, ALLEN R., Riverside, Connecticut.
MEYERHOF, OTTO, Research Professor, University of Pennsylvania School of Medicine.
NEUBERG, CARL, Research Professor, Polytechnic Institute.
PERSON, PHILIP, Research Fellow, Rutgers University.
PETTIT, LINCOLN C., Head of Biology Department, Hiram College.
ROOT, WALTER S., Professor of Physiology, College of Physicians and Surgeons.
ROYS, CHESTER, Research Associate, Tufts College.
SCHLESINGER, R. WALTER, Associate Member, Public Health Research Inst. of the City of New York.
SCHMIDT-NIELSEN, KNUT, Assistant Professor, University of Cincinnati.
SHWARTZMAN, GREGORY, Head of Dept. of Bacteriology, Mount Sinai Hospital.
STERN, KURT G., Adjunct Professor of Biochemistry, Polytechnic Institute.
SULKIN, S. EDWARD, Professor and Chairman, Dept. Bacteriology, Southwestern Medical School.
TAMM, OLGA, Assistant Librarian, The American Geographical Society of New York.
TAMM, IGOR, Assistant, Rockefeller Institute for Medical Research.
WAINIO, WALTER W., Associate Professor of Biochemistry, Rutgers University.

Students, 1951

BOTANY

DONAHUE, BARBARA ANNE, Smith College.
GROESBECK, MARJORIE E., Elmira College.
HOOPER, MARY ELIZABETH, Elmira College.
JENNINGS, PETER RANDOLPH, Drew University.
JOSEPH, T. C., Fordham University.
MASSELINK, SALLY ANN, Vassar College.
NEWHOUSE, W. JAN, University of New Hampshire.
O'BRIEN, MURIEL J., Washington, D. C.
PINKNEY, ADDISON VASTAPHA, JR., Morgan State College.
SINSKI, JAMES THOMAS, Marquette University.
SZERLIP, MARTHA, Smith College.
TREFZ, SHIRLEY M., Temple University.
WILCE, ROBERT THAYER, University of Vermont.

EMBRYOLOGY

ANDERTON, LAURA GADDES, Woman's College of the University of North Carolina.
ANGEVINE, JAY BERNARD, Cornell University.
BEHRMAN, EDWARD JOSEPH, Yale University.
BERGER, SAMUEL HAROLD, Columbia University.
BYERS, HELEN LOUISE, Indiana University.
CASTLEMAN, MARY ELIZABETH, Randolph-Macon Woman's College.
COLLIER, JACK REED, University of North Carolina.
DOSSEL, WILLIAM EDWARD, Johns Hopkins University.
FAUTEUX, MURIEL DORIS, University of Massachusetts.
FORSTHOEFEL, PAULINUS F., Ohio State University.
GALL, JOSEPH G., Yale University.
GREENE, PETER H., Amherst College.
JACOBSON, ANTONE G., Harvard College.
JENKINS, FLOYD ALBERT, St. Louis University.
LEIB, GILBERT M. P., Haverford College.
LIVINGSTONE, KATHERINE ELIZABETH, Acadia University.
MCGREGOR, INA LOUISE, Clark University.
PIAVIS, GEORGE WALTER, Westminster, Maryland.
POTTER, DAVID DICKINSON, Swarthmore College.
STEINBERG, MALCOLM SAUL, Amherst College.
SUBTELNY, STEPHEN, University of Missouri.
TAMAR, HENRY, New York University.
WATT, DONALD JAMES, Wesleyan University.
WEISHEIT, PHYLLIS JEANETTE, University of Wisconsin.
WHALLON, JEANNE, Indiana University.
WOOD, DOROTHY EVELYN, Marquette University.
YOUNGS, LILLIAN MARGOT, University of North Carolina.

PHYSIOLOGY

AMES, BRUCE NATHAN, California Institute of Technology.
BARON, JEAN BERNARD, Columbia University.
BATTLEY, EDWIN H., Stanford University.
BUDENSTEIN, ZELDA, Mount Holyoke College.
CORI, OSVALDO, University of Chile.
DAVIS, LEROY THOMAS, Syracuse University.
EGAN, RICHARD, University of Maryland School of Medicine.
EICHLER, MYRON FRANKLIN, State University of New York at New York.
ERICSON, HARTLEY CHARLES, Northwestern University.
FELDMAN, DONALD, Tulane University.
GARLINGTON, LAURENS NELSON, Duke University.
GOLLUB, SEYMOUR, Mt. Sinai Hospital.
GROTH, DONALD P., University of Wisconsin.
HALL, KENNETH DELAND, Duke University Medical School.
KEANE, JOHN FRANCIS, JR., St. Louis University.
KILLAM, IONA CLAIRE, Acadia University.
KING, DONALD W., Columbia University.
LANDAU, JOSEPH VICTOR, New York University, Washington Square College.
LEVINE, CELIA, Columbia University.
MAYO, MERCEDES DIAZ, Universidad de la Habana.
MELTON, CARLTON EARL, JR., University of Illinois.
OVERMAN, TED, Louisiana State University School of Medicine.
PYLE, ZOULA PAULINE, State University of Iowa.
RESNIK, ROBERT ALAN, Purdue University.
ROBYNS, ROSE, Rochester Medical School.
SMITHBERG, MORRIS, University of Rochester.

THURLOW, JANE ODIORNE, Rutgers University.
WALSH, RAYMOND R., Cornell University.
WELCH, CLAUDE ALTON, Michigan State College.

INVERTEBRATE ZOOLOGY

ANDERSON, PAUL KNIGHT, Orange, Massachusetts.
BACHA, WILLIAM J., JR., Long Island University.
BANKS, EDWIN M., University of Chicago.
BENNETT, MICHAEL, Yale University.
BERBERIAN, JOSEPH FRANCIS, University of Notre Dame.
BERNSTEIN, PAUL WILLIAM, Washington University.
BUCH, FLORENCE GRACE, Washington Square College.
CLARK, MARTHA GRACE, DePauw University.
COWPERTHWAITE, JEAN, New York University.
DEARDEN, LYLE C., University of Massachusetts.
FESCO, EDWARD J., Villanova College.
FLEMING, T. CORWIN, Harvard College.
FLEMINGER, ABRAHAM, Harvard University.
FLINN, LOUISE CROSWELL, Huntingdon College.
FLORI, BEATRICE MARIAN, University of Illinois.
FREDERICKSON, JEAN, Oberlin College.
GREENSTEIN, JULIUS SIDNEY, University of Illinois.
HILL, ROBERT BENJAMIN, Tufts College.
HILLS, ALICIA M., Radcliffe College.
HULL, ROBERT W., University of Illinois.
KENNEDY, DONALD, Harvard University.
KING, ELIZABETH NORFLEET, Wellesley College.
KIRNER, STEPHEN H., Hamilton College.
KURLAND, AARON, University of Illinois.
LACEY, RICHARD J., University of Illinois.
LaDAGE, BARBARA JOAN, Northwestern University.
LaFLEUR, REV. ANGELUS, Fordham University.
LEEDS, MARY M., Antioch College.
LEGAULT, REV. ROMEO O., University of Ottawa.
LOUDERMILL, PHYLLIS ANN, Hood College.
MACOMBER, KENT JEAN OAKLEY, Vassar College.
MARGEZ, ALPHONSUS, Catholic University.
MARZULLO, CARMELA MARIA, Wesleyan University.
MATHIES, ALLEN WRAY, JR., Colorado College.
McGILL, DAVID A., Bucknell University.
PARSONS, EDWARD LEWIS, University of Massachuetts.
PATERSON, MABEL, University of Illinois.
PAULSEN, ELIZABETH, Rutgers University.
PAYNE, CHARLES B., JR., Yale University.
PLAINE, HENRY LEROY, Johns Hopkins University.
PLATZ, SUSAN CARTWRIGHT, University of Illinois.
PRATT, ANNE E., Wilson College.
ROLLMAN, IRENE, Hunter College.
RONDON, TERESA, Smith College.
SACKS, MARTIN, University of Illinois.
SINDEN, MABEL, University of Minnesota.
SOHN, I. G., U. S. Geological Survey.
SPIELMAN, ANDREW, Colorado College.
STEMLER, FRED WALTER, Purdue University.
TEAL, JOHN MOLINE, Harvard University.
THIMANN, VIVIANNE, Swarthmore College.
WARREN, PATRICIA, West Newton, Massachusetts.

WOOD, EUNICE M., Wellesley College.
WORTMAN, DIANA, Brooklyn College.
YARMOLINSKY, MICHAEL BEZALEL, Johns Hopkins University.

4. THE LALOR FELLOWS, 1951

BARTON, JAY, II, Columbia University.
BENESCH, REINHOLD, Northwestern University.
CIERESZKO, LEON S., University of Oklahoma.
COHEN, SEYMOUR S., Children's Hospital of Philadelphia.
DAN, KATSUMA, Tokyo Metropolitan University.
JACOBS, WILLIAM P., Princeton University.
JØRGENSEN, C. BARKER, University of Copenhagen.
KARNOVSKY, M. L., Harvard University Medical School.
KELLY, SALLY M., Vassar College.
SCHULMAN, MARTIN P., University of Pennsylvania.

5. TABULAR VIEW OF ATTENDANCE, 1947–1951

	1947	*1948*	*1949*	*1950*	*1951*
INVESTIGATORS—Total	299	326	344	338	303
Independent	182	183	193	198	186
Under Instruction	36	42	52	43	28
Library Readers	36	50	55	48	37
Research Assistants	45			49	52
STUDENTS—Total	131	123	128	126	124
Zoology	55	54	55	55	55
Embryology	33	29	31	29	27
Physiology	26	25	27	27	29
Botany	17	15	15	15	13
TOTAL ATTENDANCE	430	449	472	444	427
Less persons registered as both students and investigators	2	6	2		
	428	443	470	444	427
INSTITUTIONS REPRESENTED—Total	148	158	155	156	158
By Investigators	114	117	114	114	115
By Students	56	68	68	67	43
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	1	1	1	2	1
By Students	1				1
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	7	8	6	6	8
By Students	3	4	3	2	3

6. COOPERATING AND SUBSCRIBING INSTITUTIONS, 1951

Cooperating Institutions

Amherst College
Brooklyn College
Bryn Mawr College
Children's Hospital of Philadelphia
College of Mt. St. Joseph-on-the-Ohio
College of Physicians and Surgeons
Columbia University
Drew University
Duke University
Elmira College
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Institute for Muscle Research

Johns Hopkins University
Johns Hopkins University Medical School
Eli Lily and Company
Massachusetts Institute of Technology
Morgan State College
Mount Sinai Hospital
National Cancer Institute
New York University College of Medicine
New York University, Heights
New York University, Washington Square College
North Carolina State College of Agriculture and Engineering
Northwestern University
Oberlin College
Princeton University
Rockefeller Institute for Medical Research
Rutgers University
Saint Louis University
Smith College
Southwestern Medical College
State College of New York, School of Medicine
Temple University
Tufts College Medical School
Union College
University of Chicago
University of Connecticut
University of Delaware
University of Illinois
University of Kansas
University of Maryland School of Medicine
University of Massachusetts
University of Michigan
University of Minnesota
University of Missouri
University of New Hampshire
University of Oklahoma
University of Pennsylvania
University of Pennsylvania Medical School
University of Vermont Medical School
University of Virginia
University of Wisconsin
Vassar College
Washington University
Wesleyan University
Western Reserve University
Yale University

Subscribing Institutions

Acadia University
Catholic University
Hahnemann Medical School
Indiana University
Jefferson Medical College
Long Island University
Marquette University
National Research Council
Public Health Research Institute of New York City
Radcliffe College
Tulane University School of Medicine
University of North Carolina
University of Rochester
University of South Dakota
Wellesley College

7. Evening Lectures

The Friday Evening Lectures, 1951

Friday, June 29
Dr. Douglas A. Marsland ..... "Gelation in Relation to Protoplasmic Contractility."

Friday, July 6
Dr. Severo Ochoa ..... "Carbon Dioxide Fixation in Animals and Plants."

Friday, July 13
Dr. Ralph W. Gerard ..... "Neural Basis of Behaviour."

Friday, July 20
Dr. Balduin Lucké ..... "Tumors in Cold-blooded Vertebrates. Their Significance in the Experimental Study of Cancer."

Friday, July 27
Dr. Hans Borei ..... "Respiration of the Egg Cell Before and After Fertilization."

Friday, August 3
Dr. Denis L. Fox ..... "Colloidal Materials in the Nutrient Cycle of the Sea."

Friday, August 10
Dr. Nelson T. Spratt, Jr. ..... "Nutritional and Environmental Requirements for Development of the Early Chick Embryo."

Friday, August 17
Dr. T. H. Waterman ..... "The Physiology of the Compound Eye."

Friday, August 24
Dr. R. W. G. Wyckoff ..... "Macromolecular Particles and Viruses in Tissues of Plants and Animals."

Other Lectures

Monday, August 13
Dr. Katsuma Dan ..... "Cyto-embryology in the Sea Urchin."

8. Seminars, 1951

July 3
B. Libet, H. J. Ralston and B. Feinstein ..... "Effect of Stretch on Action Potentials of Muscle."
L. S. Ciereszko ..... "An Application of Ion-Exchange Resins to the Isolation of Amino Acids from Biological Materials."
J. T. Bonner ..... "Morphogenesis of the Slime Bacterium, *Chondromgees.*"

July 10
H. S. Mason and E. Davis ..... "Status of Luciferin Chemistry."
Claude A. Villee and Dorothy Balzer ..... "Studies of the Metabolism of Fetal and Placental Tissues."
Wm. P. Jacobs ..... "The Nature of the Hormonal Factor Which Normally Limits the Differentiation of a Specific Cell-Type."

July 17
Reinhold Benesch and Ruth E. Benesch ..... "Studies on Biologically Occurring Mercaptans and Some of Their Mercaptides."
Charles G. Wilber ..... "Blood and Liver Lipids in the Northern Pike."
Gerrit Bevelander ..... "Calcification in Molluscs."

July 24
Sidney Solomon and Julian Tobias .. "Long Axis Movement of Nerve Ca, K, and Na in an Electric Field."
Rudi Oberholzer ..... "Influence of Various Potassium Concentrations on Resting Respiration of Excised Frog Nerve."
I. B. Wilson ..... "Reaction of Substrates and Inhibitors With the Surface of Acetylcholinesterase."

D. NACHMANSOHN ..... "The Curare Effect and Axonal Conduction."

July 31

IVOR CORNMAN ..... "Specificity of Steroids Which Inhibit Heart Beat."

EDWARD G. BOETTIGER AND E. FURSHPAN ..... "Observations of the Flight Mechanism of Flies."

DAVID R. STADLER ..... "Chemotropism in *Rhizopus nigricans:* The Staling Reaction."

9. MEMBERS OF THE CORPORATION, 1951

1. LIFE MEMBERS

BECKWITH, DR. CORA J., Vassar College, Poughkeepsie, New York.
BILLINGS, MR. R. C., 66 Franklin Street, Boston, Massachusetts.
CALVERT, DR. PHILIP P., University of Pennsylvania, Philadelphia, Pennsylvania.
COLE, DR. LEON J., College of Agriculture, Madison, Wisconsin.
CONKLIN, PROF. EDWIN G., Princeton University, Princeton, New Jersey.
COWDRY, DR. E. V., Washington University, St. Louis, Missouri.
JACKSON, MR. CHAS. C., 24 Congress Street, Boston, Massachusetts.
JACKSON, MISS M. C., 88 Marlboro Street, Boston, Massachusetts.
KING, MR. CHAS. A.
LEWIS, PROF. W. H., Johns Hopkins University, Baltimore, Maryland.
MACNAUGHT, FRANK M., Woods Hole, Massachusetts.
MEANS, DR. J. H., 15 Chestnut Street, Boston, Massachusetts.
MOORE, DR. GEORGE T., Missouri Botanical Gardens, St. Louis, Missouri.
MOORE, DR. J. PERCY, University of Pennsylvania, Philadelphia, Pa.
MORGAN, MRS. T. H., Pasadena, California.
NOYES, MISS EVA J.
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
SCOTT, DR. ERNEST L., Columbia University, New York City, New York.
SEARS, DR. HENRY F., 86 Beacon Street, Boston, Massachusetts.
SHEDD, MR. E. A.
STRONG, DR. O. S., Columbia University, New York City, New York.
WAITE, PROF. F. C., 144 Locust Street, Dover, New Hampshire.
WALLACE, LOUISE B., 359 Lytton Avenue, Palo Alto, California.

2. REGULAR MEMBERS

ADAMS, DR. A. ELIZABETH, Mount Holyoke College, South Hadley, Massachusetts.
ADDISON, DR. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
ADOLPH, DR. EDWARD F., University of Rochester Medical School, Rochester, New York.
ALBAUM, DR. HARRY G., Biology Dept., Brooklyn College, Brooklyn, N. Y.
ALBERT, DR. ALEXANDER, Mayo Clinic, Rochester, Minnesota.
ALLEE, DR. W. C., 114 Leigh Hall, University of Florida, Gainesville, Florida.

AMBERSON, DR. WILLIAM R., Department of Physiology, University of Maryland, School of Medicine, Baltimore, Md.

ANDERSON, DR. RUBERT S., Department of Physiology, University of South Dakota, Vermillion, South Dakota.

ANDERSON, DR. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.

ANGERER, PROF. C. A., Department of Physiology, Ohio State University, Columbus, Ohio.

ARMSTRONG, DR. P. B., State College of New York Medical Center at Syracuse, New York.

ATWOOD, DR. KIMBALL C., 68½ Outer Drive, Oak Ridge, Tennessee.

AUSTIN, DR. MARY L., Wellesley College, Wellesley, Massachusetts.

BAITSELL, DR. GEORGE A., Yale University, New Haven, Connecticut.

BAKER, DR. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.

BALL, DR. ERIC G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.

BALLARD, DR. WILLIAM W., Dartmouth College, Hanover, New Hampshire.

BARD, PROF. PHILIP, Johns Hopkins Medical School, Baltimore, Maryland.

BARRON, DR. E. S. GUZMAN, Department of Medicine, University of Chicago, Chicago, Illinois.

BARTH, DR. L. G., Department of Zoology, Columbia University, New York City, New York.

BARTLETT, DR. JAMES H., Department of Physics, University of Illinois, Urbana, Illinois.

BEAMS, DR. HAROLD W., Department of Zoology, State University of Iowa, Iowa City, Iowa.

BECK, DR. L. V., Department of Physiology and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh 13, Pennsylvania.

BEERS, DR. C. D., University of North Carolina, Chapel Hill, North Carolina.

BEHRE, DR. ELINOR H., Louisiana State University, Baton Rouge, Louisiana.

BERTHOLF, DR. LLOYD M., College of the Pacific, Stockton, California.

BEVELANDER, DR. GERRIT, New York University School of Medicine, New York City, New York.

BIGELOW, DR. H. B., Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts.

BIGELOW, PROF. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.

BISHOP, DR. DAVID W., Department of Physiology, University of Massachusetts, Amherst, Massachusetts.

BISSONNETTE, DR. T. HUME, Trinity College, Hartford, Connecticut.

BLANCHARD, PROF. K. C., Johns Hopkins Medical School, Baltimore, Maryland.

BLUM, DR. HAROLD F., Department of Biology, Princeton University, Princeton, New Jersey.

BODANSKY, DR. OSCAR, Clinical Pharmacology, Cornell University Medical College, New York City, New York.

BODIAN, DR. DAVID, Department of Epidemiology, Johns Hopkins University, Baltimore, Maryland.

BODINE, DR. J. H., Department of Zoology, State University of Iowa, Iowa City, Iowa.
BOELL, DR. EDGAR J., Yale University, New Haven, Connecticut.
BOETTIGER, DR. EDWARD G., Department of Zoology, University of Connecticut, Storrs, Connecticut.
BONNER, DR. JOHN T., Department of Biology, Princeton University, Princeton, New Jersey.
BRADLEY, PROF. HAROLD C., 2639 Durant Avenue, Berkeley 4, California.
BRODIE, MR. DONALD M., 522 Fifth Avenue, New York City, New York.
BRONFENBRENNER, DR. JACQUES J., Department of Bacteriology, Washington University Medical School, St. Louis, Missouri.
BRONK, DR. DETLEV W., Johns Hopkins University, Baltimore, Maryland.
BROOKS, DR. MATILDA M., University of California, Department of Zoology, Berkeley, California.
BROWN, DR. DUGALD E. S., Department of Zoology, University of Michigan, Ann Arbor, Michigan.
BROWN, DR. FRANK A., JR., Department of Biological Sciences, Northwestern University, Evanston, Illinois.
BROWNELL, DR. KATHERINE A., Ohio State University, Columbus, Ohio.
BUCK, DR. JOHN B., Industrial Hygiene Research Lab., National Institute of Health, Bethesda, Maryland.
BUCKINGHAM, MISS EDITH N., Sudbury, Massachusetts.
BUDINGTON, PROF. R. A., Box 954, Winter Park, Florida.
BULLINGTON, DR. W. E., Randolph-Macon College, Ashland, Virginia.
BULLOCK, DR. T. H., University of California, Los Angeles 24, California.
BURBANCK, DR. WILLIAM D., Box 834, Emory University, Georgia.
BURDICK, DR. C. LALOR, The Lalor Foundation, Lancaster Pike and Old Baltimore Rd., Wilmington, Delaware.
BURKENROAD, DR. M. D., Institute of Marine Science, Port Arkansas, Texas.
BUTLER, DR. E. G., Princeton University, Princeton, N. J.
CAMERON, DR. J. A., Baylor College of Dentistry, Dallas, Texas.
CANNAN, PROF. R. K., New York University College of Medicine, New York City, New York.
CANTONI, DR. GIULIO, Department of Pharmacology, Western Reserve University, Cleveland 9, Ohio.
CARLSON, PROF. A. J., Department of Physiology, University of Chicago, Chicago, Illinois.
CAROTHERS, DR. E. ELEANOR, 134 Avenue C. East, Kingman, Kansas.
CARPENTER, DR. RUSSELL L., Tufts College, Tufts College, Massachusetts.
CARVER, PROF. GAIL L., Mercer University, Macon, Georgia.
CATTELL, DR. MCKEEN, Cornell University Medical College, New York City, New York.
CATTELL, MR. WARE, Cosmos Club, Washington, D. C.
CHAMBERS, DR. EDWARD L., Department of Anatomy, Johns Hopkins University, Baltimore, Maryland.
CHAMBERS, DR. ROBERT, Dade County Cancer Institute, Miami, Florida.
CHARLES, DR. DONALD R., Department of Zoology, Division of Biological Sciences, University of Rochester, Rochester 3, New York.

CHASE, DR. AURIN M., Princeton University, Princeton, New Jersey.
CHENEY, DR. RALPH H., Biology Department, Brooklyn College, Brooklyn 10, New York.
CHILD, PROF. C. M., Jordan Hall, Stanford University, California.
CHURNEY, DR. LEON, Department of Physiology, Louisiana State University School of Medicine, New Orleans 13, Louisiana.
CLAFF, MR. C. LLOYD, 5 Van Beal Road, Randolph, Massachusetts.
CLARK, DR. A. M., Department of Biology, University of Delaware, Newark, Delaware.
CLARK, PROF. E. R., Wistar Institute, Woodland Avenue and 36th Street, Philadelphia 4, Pennsylvania.
CLARK, DR. LEONARD B., Department of Biology, Union College, Schenectady, New York.
CLARKE, DR. G. L., Department of Biology, Harvard University, Cambridge 38, Mass.
CLELAND, PROF. RALPH E., Indiana University, Bloomington, Indiana.
CLEMENT, DR. A. C., Department of Biology, Emory University, Emory, Georgia.
CLOWES, DR. G. H. A., Eli Lilly and Company, Indianapolis, Indiana.
COE, PROF. W. R., Scripps Institute of Oceanography, La Jolla, California.
COHEN, DR. SEYMOUR S., Department of Physiological Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania.
COHN, DR. EDWIN J., 183 Brattle Street, Cambridge, Massachusetts.
COLE, DR. ELBERT C., Department of Biology, Williams College, Williamstown, Massachusetts.
COLE, DR. KENNETH S., Naval Medical Research Institute, Bethesda 14, Maryland.
COLLETT, DR. MARY E., Western Reserve University, Mather College, Cleveland, Ohio.
COLTON, PROF. H. S., Box 601, Flagstaff, Arizona.
COLWIN, DR. ARTHUR L., Department of Biology, Queens College, Flushing, Long Island, New York.
COLWIN, DR. LAURA N. H., Department of Biology, Queens College, Flushing, Long Island, New York.
COOPER, DR. KENNETH W., Department of Biology, Princeton University, Princeton, New Jersey.
COOPERSTEIN, DR. SHERWIN J., Department of Anatomy, Western Reserve University Medical School, Cleveland, Ohio.
COPELAND, DR. D. E., 1027 North Manchester Street, Arlington 5, Virginia.
COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Maine.
COPLEY, DR. ALFRED L., Flower Hospital, 5th Avenue, New York City, New York.
CORNMAN, DR. IVOR, George Washington University, Warwick Memorial Clinic, Washington 5, D. C.
COSTELLO, DR. DONALD P., Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.
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THE EFFECTS OF DIFFERENT HALOGENATED ALKYL AMINES ON THE DIVISION OF SEA URCHIN EGGS[1]

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With a view to obtaining some insight into the cytological effects of a representative series of nitrogen mustards, 27 compounds, mostly β-chloroethyl amines, were tested for their ability to block or retard the cleavage of sea urchin eggs.

With such eggs, each exposed directly to the experimental solution, there is unexcelled opportunity to study the response of the cell to the mustard without concern for effects of a multicellular organism on the mustard, and on the response of the cell. With regard to mitosis, the uniform, controllable division of the eggs makes possible the application of the agent to any phase of cleavage, and ready analysis of alterations in each phase.

Materials and Methods

For the over-all survey, the eggs of *Arbacia punctulata* were used. Some supplementary studies employed the eggs of *Tripneustes esculentus*, and for detailed observations of the course of events during inhibition of mitosis, the transparent eggs of *Lytechinus variegatus* were used.

For routine comparison of activities, exposure was begun 10–13 minutes after fertilization. Retardation was measured by making a count of 50 eggs 4 to 10 times during the first cleavage and again during the second. The points were connected by straight lines to determine the approximate time of 50 per cent cleavage (*cf.* Fig. 1), and comparison was made between the treated and the control from the same lot of eggs.

Curves for duplicate controls usually gave identical 50 per cent cleavage times, and they seldom differed by more than two minutes, so statistical analysis was not needed. The eggs were followed until they formed plutei, and a record was kept of the time at which they became blastulae and gastrulae. The ranges of response were condensed into groups described in connection with Table II.

The compounds (Table I) were dissolved in sea water and were added to the eggs within two minutes of dissolving. They were supplied to the Sloan-Kettering Institute by Parke-Davis and Company (A); Toxicity Laboratory, The University of Chicago (B); Dr. Stein, Rockefeller Institute (C); Merck and Company (D); Eli Lilly and Company (E); and American Cyanamid Company (F).

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Results and Discussion

Arbacia

The nature of the response of dividing eggs to nitrogen mustard at moderately effective doses is shown in Figure 1. Lower concentrations caused less retardation or affected only the second division or even later stages, while higher concentrations prevented division in all or part of the population.

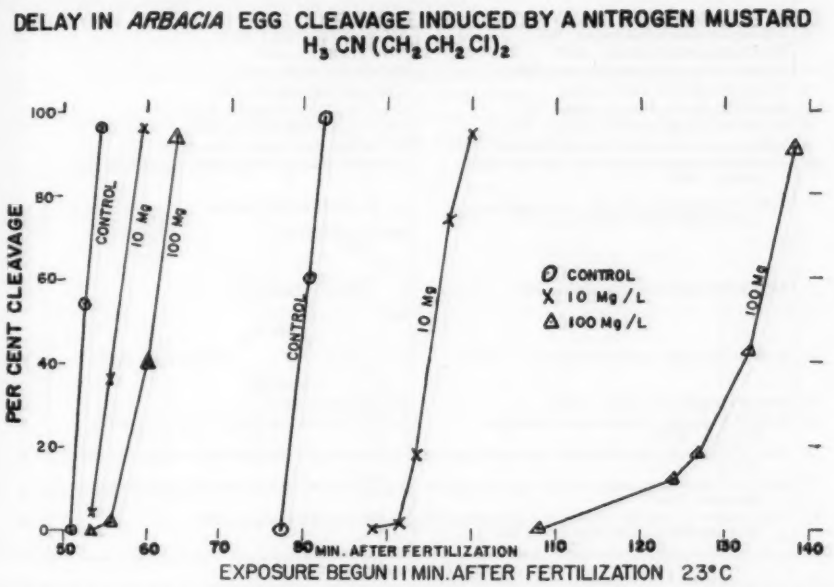
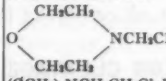
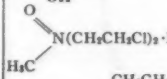
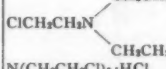
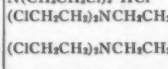
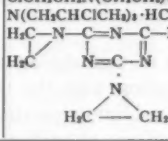
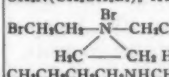


Figure 1. The three curves on the left represent first cleavage, the others second cleavage.

The different degrees of retardation and blocking of Arbacia-egg cleavage are shown in Table II, beginning with the most active mustard at the top. Concentrations are expressed as millimols per liter. The grades of effect are the same as those used to quantitate effects of carbamates (Cornman, 1950). Where development was not completely blocked, retardation of the first cleavage was determined from graphs like Figure 1 and then placed in one of three grades: 100% or more, 10% to 99%, or less than 10% increase in the time required to reach 50% first cleavage as compared with the controls. Weaker effects were detected as retardation of second cleavage or of embryogenesis. At 23° ± 0.5° C., the temperature used for all Arbacia experiments, 50 per cent first cleavage was reached usually at 52–53 minutes, but in one experiment was as early as 50 minutes and in another as late as 61 minutes. Second cleavage reached 50 per cent at 84–85 minutes, with extremes at 80 and 93 minutes.

TABLE I

Names and structures of compounds studied

No.	Name	Structure	Source
		β-chloroethylamines	
1	*β*-chloroethyl amine. HCl	$NH_2CH_2CH_2Cl \cdot HCl$	A
2	N-(*β*-chloroethyl)-morpholine		B
3	Dibenzyl-*β*-chloroethyl amine. HCl	$(\emptyset CH_2)_2NCH_2CH_2Cl \cdot HCl$	B
4	*Bis*-(*β*-chloroethyl)-amine. HCl	$NH(CH_2CH_2Cl)_2 \cdot HCl$	A
5	Methyl-*bis*-(*β*-chloroethyl)-amine. HCl	$H_3CN(CH_2CH_2Cl)_2 \cdot HCl$	B
6	Propyl-*bis*-(*β*-chloroethyl)-amine	$CH_3CH_2CH_2N(CH_2CH_2Cl)_2$	B
7	*n*-amyl-*bis*-(*β*-chloroethyl)-amine. HCl	$CH_3(CH_2)_4N(CH_2CH_2Cl)_2 \cdot HCl$	D
8	*n*-hexyl-*bis*-(*β*-chloroethyl)-amine. HCl	$CH_3(CH_2)_5N(CH_2CH_2Cl)_2 \cdot HCl$	D
9	*γ*-phenylpropyl-*bis*-(*β*-chloroethyl)-amine. HCl	$\emptyset CH_2CH_2CH_2N(CH_2CH_2Cl)_2 \cdot HCl$	D
10	*p*-octylphenoxyethoxyethyl-*bis*-(*β*-chloroethyl)-amine. HCl	$C_8H_{17}\emptyset OCH_2CH_2OCH_2CH_2N(CH_2CH_2Cl)_2 \cdot HCl$	D
11	3-*bis*-(*β*-chloroethyl)-aminom hyl-4-methoxy-methyl-5-hydroxy-6-methyl-pyridine. 2HCl	 2HCl	D
12	Methyl-*bis*-(*β*-chloroethyl)-amine oxide. HCl		D
13	N,N′-*bis*-(*β*-chloroethyl)-1,4-piperazine. HCl		D
14	*Tris*-(*β*-chloroethyl)-amine. HCl	$N(CH_2CH_2Cl)_3 \cdot HCl$	B
15	N,N,N′,N′-*tetrakis*-(*β*-chloroethyl)-ethylene-diamine. 2HCl	$(ClCH_2CH_2)_2NCH_2CH_2N(CH_2CH_2Cl)_2 \cdot 2HCl$	B
16	N,N,N′,N′-*tetrakis*-(*β*-chloroethyl)-propane-diamine. 2HCl	$(ClCH_2CH_2)_2NCH_2CH_2CH_2N(CH_2CH_2Cl)_2 \cdot 2HCl$	B
17	N,N,N′,N′-*tetrakis*-(*β*-chloroethyl)-*β*-chloropropane-diamine. 2HCl	$(ClCH_2CH_2)_2NCH_2CHClCH_2N(CH_2CH_2Cl)_2 \cdot 2HCl$	B
18	Composition uncertain. Probably a polymer of *bis*-(*β*-chloroethyl)-amine with M.W. 764	$H_2[HCN(CH_2CH_2Cl)_2]_n \cdot nHCl$	D
		Other Chloroalkylamines	
19	Ethyl-*γ*-chloropropyl-*β*-chloroethylamine. HCl	$ClCH_2CH_2N(CH_2CH_3)CH_2CH_2CH_2Cl \cdot HCl$	E
20	*Tris*-(*β*-chloro-*n*-propyl)-amine. HCl. $\frac{1}{2}H_2O$	$N(CH_2CHClCH_3)_3 \cdot HCl \cdot \frac{1}{2}H_2O$	D
21	Triethylenimino-*s*-triazine		F
		Other Haloalkylamines	
22	Methyl-*bis*-(*β*-bromoethyl)-amine. HBr	$CH_3N(CH_2CH_2Br)_2 \cdot HBr$	D
23	N,N′-(*β*-bromoethyl)-N,N′-(*β*-ethylene-imonium-bromide)-ethylene-diamine		D
24	*n*-butyl-*β*-fluoroethylamine. HCl	$CH_3CH_2CH_2CH_2NHCH_2CH_2F \cdot HCl$	D
25	*n*-butyl-*bis*-(*β*-fluoroethyl)-amine. HCl	$CH_3CH_2CH_2CH_2N(CH_2CH_2F)_2 \cdot HCl$	D
		Substituted Ammonium Chlorides	
26	*bis*-(*β*-chloroethyl)-dimethyl-ammonium chloride	$(CH_3)_2NCl(CH_2CH_2Cl)_2$	B
27	tetra-(*β*-chloroethyl)-ammonium chloride	$(ClCH_2CH_2)_4NCl(CH_2CH_2Cl)_3$	C

This series of mustards divides roughly into two groups, the majority blocking cleavage at a few millimols per liter, and retarding the first division at the tenths or hundredths millimolar level (down to No. 13, Table II). About half of this group, including the more typical nitrogen mustards (8, 15, 7, 9, 14, 6, 22), are somewhat more active than the others, increasing cleavage time 100 per cent or more at 0.3–0.5 mM/L and 10–99 per cent at 0.03–0.05 mM/L. The quick effectiveness of *n*-hexyl-*bis*-(β-chloroethyl)-amine (No. 8), preventing all division at 0.38 mM/L, suggests that the six-carbon chain is about optimal for penetration, as against the longer and shorter groups tested.

TABLE II

Relative potencies (mM/L) of halogenated alkyl amines in preventing or retarding Arbacia egg cleavage

Compound No.	Millimolarity required to induce:						
	Complete block (Aa)	Retardation					No effect
		First cleavage			Second cleavage (Cd)	Embryo (D)	
		100% (Ca)	10–99% (Cb)	<10% (Cc)			
8	0.38		.038	.019 .008			
15	2.61	.261	.026				
7	4.02	.402	.040				
9	3.37	.337	.034				
14	4.15	.415	.042				
6	5.44	.544	.544	.054			
22	3.07		.307 .031				
20	3.43		.343	.034			
12			.481	.048			.005
5	5.21		.521	.052			
19			4.52 .452	.045			
10	2.20			.220	.022		
18		1.31		.131	.013		
13	4.05				.405		
3				.338			.034
2			6.85	.685			
1			8.62	.862 .086			
27			3.29				.329
4			11.17	5.59 .559 .056			
26						4.83 .483	
23			20.49				2.05

Aa: No nuclear or cytoplasmic division. C: The time between fertilization and 50% first cleavage increased by 100% (Ca), by 10–99% (Cb), or by less than 10% (Cc). Cd: First cleavage not retarded but second cleavage retarded. D: First two cleavages not retarded but the blastula, gastrula, or pluteus retarded.

Two potent mustards deviate from the typical structural pattern. Methyl-*bis*-(β-chloroethyl)-amine oxide (No. 12) has its nitrogen occupied by oxygen in addition to the alkyl chains. Attachment of the β-chloroethyl radicals to separate nitrogens (No. 13) also forms an active molecule, as pointed out by Goldacre, Loveless and Ross (1949). A terminal chlorine (No. 19) or a β-chlorine (No. 20) in a propyl chain appears to function as well as an ethyl β-chlorine, although Haddow, Kon and Ross (1948) find the γ chlorine inactive in chloroalkylaryl amines. The poor performance of No. 4 is under suspicion, particularly since these compounds hydrolyze readily in the moist seaside atmosphere. A fresh sample was not available for re-checking.

Mustards with only a single β-chloroethyl group (Nos. 1, 2, 3) are relatively inactive, as reported by Haddow, Kon and Ross (1948). On the other hand, addition of a third β-chloroethyl group (14) or even a second substituted nitrogen (15) does not confer outstanding potency on the molecule.

Tripneustes

Experiments with another species at the same temperature show some differences in response, and, because the egg is unpigmented, yield somewhat more in the way of cytological detail.

For comparison, the column headings used for Arbacia (Table II: Ca, Cb, etc., indicating the extent of retardation) are similarly used for Tripneustes in Table III. An additional category, *Incomplete block*, encompasses more severe effects where less than 5 per cent of the eggs divided (b) or where they did not divide at all, but unlike completely blocked eggs, they formed temporary furrows or underwent nuclear division in the absence of cytoplasmic division (Ba). Comparison is made at the time of 50 per cent cleavage which was at 86–101 minutes for the first, and 131–182 minutes for the second division at 23°, or 65–82 minutes for the first and 101–122 minutes for the second at 25°–26° C.

Some distinctive cytological responses were observed in the living eggs. In 0.521 mM of methyl-*bis*-(β-chloroethyl)-amine (No. 5), asters and spindle remained distinct during the 1½ hours that cleavage was delayed and persisted even longer in eggs which did not divide, eventually producing tetranucleate eggs at 6 hours. At 0.052 mM, 60% of the eggs furrowed, but the furrows retracted in half of these. Persistent, often multiple, mitotic figures were also seen in compounds 15 and 24.

In comparing the effects of Nos. 5, 14 and 15 with the less severe damage to Arbacia eggs, it should be kept in mind that the length of exposure before the first cleavage is nearly twice as long in Tripneustes, which divides at about 1½ hours as against 50–60 minutes for Arbacia at 23° C.

No. 24 has only a single fluoroethyl radical, and, as we have seen from Arbacia studies, such "hemi-mustards" are relatively ineffective. However, No. 24, although having both β-fluoroethyl groups, is considerably less toxic than the chloroethylamines. The difference cannot as yet be assigned entirely to the halogen inasmuch as the first five (14, 15, 16, 17, 5) were tested at Bermuda at 23° C. and the last three (11, 25, 24) at Bimini at 25°–26° C. There are also differences among different experiments with the same compound, as shown by diverse severity of effects from identical concentrations of Nos. 15 and 24. These dif-

ferences arise from differences in the numbers of eggs in separate experiments and probably from differences in the individual urchins.

Lytechinus

In this species 50 per cent cleavage is reached at 46–61 minutes and the second at 70–83 minutes at 25°–27° C.

Triethylenimino-s-triazine

Triethylene melamine is a name commonly applied to compound No. 21. Although lacking the β-halogen, it already carries the imine rings which are formed by some chloroethylamines in solution (Golumbic *et al.*, 1946) and it resembles nitrogen mustards at least in its ability to retard leukemia (Burchenal, *et al.*, 1950) and solid tumors (Lewis and Crossley, 1950), and its effects on cell division (Rose, Hendry and Walpole, 1950).

TABLE III

Relative potencies (mM/L) of halogenated alkyl amines in preventing or retarding Tripneustes egg cleavage

Compound No.	Millimolarity required to induce:								
	Block			Retardation					No effect
	Complete (Aa)	Incomplete		First cleavage			Second cleavage (Cd)	Embryo (D)	
		(Ba)	(Bb)	100% (Ca)	10–99% (Cb)	<10% (Cc)			
14	0.415			0.042		0.004			0.0004
15		0.261		0.261	0.026	0.026 0.003			0.003 0.0003
16			0.252		0.025 0.003				0.0002
17					0.232 0.023		0.002		0.0002
5				0.521	0.052	0.005			0.0005
11	2.630				0.500 0.263 0.250		0.025		
25					4.975	0.498		0.050	
24					6.430	0.643	6.430	0.064	

Aa: No cytoplasmic or nuclear division. Ba: Evanescent furrows or nuclear division in the absence of cytoplasmic division. Bb: Less than 5% of the eggs divided. C: The time between fertilization and 50% first cleavage increased by 100% (Ca), by 10–99% (Cb), or by less than 10% (Cc). Cd: First cleavage not retarded but second cleavage retarded. D: First two cleavages not retarded but the blastula, gastrula, or pluteus retarded.

At 9.8 mM/L it retarded the first cleavage 35–42 minutes (Cb) and prevented completion of the second cleavage. Half this dose retarded the first cleavage 25–30 minutes (Cb) and the second cleavage 1–2 hours. The second division was remarkably unequal, producing some blastomeres ¼ and ⅓ the diameter of their sisters. At 0.49 mM/L the first cleavage was retarded 4 minutes and the second 29

minutes, but the embryos died before becoming blastulae. Retardation of the first cleavage was one minute and the second 9 minutes at 0.049 mM/L. These became abnormal gastrulae.

Triethylenimino-*s*-triazine thus resembles the mustards in producing extreme retardation at comparable doses, but is unlike them in that concentrations which produce slight retardations are eventually lethal.

Dose-effect relationships with HN2

For the more detailed study of dose-effect relations and cytological effects, methyl-*bis* (β-chloroethyl)-amine (Compound 5, often called HN2) was chosen as a typical mustard. The Lytechinus egg was used because the nucleus and mitotic figure can be clearly observed in the living egg.

TABLE IV

Relationships between concentration of HN2 and inhibition of cleavage in Lytechinus eggs

	mM/L	% First cleavage		Retardation (minutes)		% Increase in cleavage time	
		Count	% Inhib.	1st cl.	2nd cl.	1st cl.	2nd cl.
Expt. III	8.320	2	98				
	4.160	22	76				
	2.080	46	49	40	210	63	248
	1.040	68	24	32	70	50	83
Expt. I	2.080	36	60	38	180	68	254
	1.040	90	0	9½	54½	17	66
	0.521			4	37	7	45
	0.260			0	21		25
	0.130			0	15½		19
	0.065			0	7		8
	0.032			0	1½		2

Expt. III: Control first cleavage 63½ min.; second cleavage 84½ min.
Expt. I: Control first cleavage 55½ min.; second cleavage 82½ min.
Percentage inhibition based on 90% control cleavage in both experiments.

The percentages of Table IV show that the curve for blocking (2–8 mM) is steeper than for retardation (0.03–1.0 mM), suggesting that two mechanisms are involved. Quite possibly cytoplasmic division is affected separately at the higher concentrations, a separation which was visible in eggs where cleavage was suppressed but where the achromatic figure was still active and the nucleus sometimes divided. Higher doses were required to produce effects comparable to those obtained with Arbacia and Tripneustes. These experiments were run at Bimini at temperatures of 24.7°–25.8° C., so environmental as well as species differences may enter in.

"Prophase block"

Some investigators find that the prophase is particularly susceptible to mustard and other "radiomimetic" poisons. In sea urchin eggs, no one phase appeared

to be selectively hit. Rather, the entire mitotic sequence was slowed. In the experiments described below, the average temperature ranged from 24.7° C. to 26.7° C., with a maximum variation within each experiment of 0.2° C.

Eggs heavily poisoned (8.32 mM/L) 44–53 minutes before control 50 per cent cleavage, *i.e.*, early prophase, maintained a high percentage of intact nuclei, although the number fluctuated as if the nuclei were dissolved and reforming. In experiment III the first drop in numbers of nuclei was preceded by a period of persistent asters. The second decline, at 13 hours, showed no evidence of mitotic activity other than the disappearance of half of the nuclei—which then reappeared by 22 hours. At slightly lower doses (6.25 and 4.16 mM/L) an appreciable number of eggs divided, preceded by a normal dissolution of the nucleus. But nuclei also disappeared about the same time in 25–75 per cent of the eggs which did not divide. Moreover nuclei reappeared in only 25–75 per cent of the divided eggs. Hughes (1950) also reports reconstruction of nuclei in cells blocked at metaphase by nitrogen mustard.

Exposure begun when the eggs were in late prophase or in metaphase retarded the first cleavage, but permitted most of the eggs to divide. At 8.32, 6.25 and 4.16 mM/L the effects were the same as with doses earlier in the cycle, except that they appeared one cleavage later. Nuclei appeared in some of the blastomeres, and some of these then disappeared, usually coincident with cleavage. Most of the blastomeres resulting from the second and later cleavages did not form nuclei. A single nucleus appeared in about half of the eggs which did not divide. A small percentage of the one-cell and two-cell stages formed karyomeres instead of whole nuclei. Occasionally a blastomere became binucleate or underwent multiple cleavage.

Exposure to 6.25 mM/L at the end of the first mitotic cycle or early in the second produced the same phenomena one stage later. Nuclei appeared in most of the blastomeres. Half of them disappeared at a time when other eggs were fragmenting, and in experiment VI, at least (exposure 17 minutes before 50 per cent second cleavage), most of these nuclei had reappeared by 9 hours after fertilization. At the two-cell and four-cell stages, karyomeres appeared in from 6 to 100 per cent of the blastomeres, depending upon the experiment. Rarely a blastomere at the two-cell stage was binucleate. Exposure begun when some of the eggs had already divided twice yielded a nuclear cycle in the four-cell stage: nuclei reaching 64 per cent, then dropping to 34 per cent as some of the eggs divided, and finally disappearing from all eggs at 9½ hours, when a fourth of the eggs were disintegrating.

These data show that even when eggs are exposed in early prophase, and poisoned to the extent that the prophase is much prolonged, the nucleus nevertheless dissolves and often re-forms. These reformed nuclei may be the "prophases" observed by others. Exposure late in the mitotic cycle shows that eggs can be stopped after completion of the prophase, presumably at metaphase since subsequently many form a single nucleus. Karyomeres, binucleation and multiple cleavage testify to the varying degrees of disruption to which the telophase is subject.

If there were a prophase effect such as is found in the intact mammal, one would expect that at the time for the first prophase, or at least at the second prophase, the nucleus would fold up into a pycnotic mass and the whole cell would expire. Yet, the prophases appear in an orderly manner, the asters build up, and a spindle replaces the nucleus. Such a mitotic figure might persist for an hour and

a half, and eventually succeed in dividing the egg. Indeed, if any one phase is to be considered sensitive in the sea-urchin egg, it is telophase. Frequently the eggs almost divide and then the furrow regresses, leaving a binucleate egg.

Evidence for prophase damage by mustards comes mostly from experiments with intact animals (Dustin, 1947; Friedenwald and Buschke, 1948; Gillette and Bodenstein, 1946). It is not found in isolated cells such as Arbacia eggs (Hutchens and Podolsky, 1948) or tissue cultures (Hughes, 1950; Meier and Schär, 1947; Fell and Allsopp, 1949) or in some plants (Novick and Sparrow, 1950; Hohl, 1948). This suggests that systemic influences on mitosis be considered before different sensitivities of division phases be ascribed entirely to intrinsic properties of the mitotic cycle.

Synergism with urethan

Skipper (1949) has found a synergism between nitrogen mustards and urethan in decreasing the white cell count in mice. For comparison, eggs were exposed to 22.5 and 45 mM urethan, and 0.032 and 0.065 mM HN2 (No. 5) separately or combined.

Urethan alone at 22.5 mM retarded first cleavage 6 minutes, and second cleavage 6 minutes and retarded the gastrulae. HN2 alone at 0.032 mM retarded the second cleavage 1½ minutes and caused abnormal blastulae. Together they retarded the first cleavage one-half minute and the second cleavage three minutes and produced abnormal blastulae.

In urethan alone at 45 mM, only 12 per cent of the eggs had divided an hour after the controls divided, and the gastrulae were irregular. HN2 at 0.065 mM did not affect the first cleavage, but retarded the second 7 minutes, and produced abnormal blastulae. These doses combined gave 12 per cent cleavage an hour late, and irregular blastulae.

The effects are no more than the separate effects of the urethan on the early cleavages and HN2 on the larvae. The synergism observed in mice would appear to involve systemic effects which do not operate on isolated dividing cells. Further, the different patterns of effect and of dose-effect ratios indicate caution (already pointed out by Loveless and Revell, 1949) in grouping these poisons together as "radiomimetic" (Dustin, 1947).

Summary

1. The capacities of different mustards for blocking or delaying the cleavage of sea urchin eggs were compared by exposing the eggs of *Arbacia punctulata, Tripneustes esculentus* and *Lytechinus variegatus* to freshly prepared solutions continuously, beginning 10 to 13 minutes after fertilization.

2. At least two β-halo groups are necessary to confer the highest potency of the molecule. A third β-chloroethyl or a second *bis*-(β-chloroethyl)-amine group, or a non-chlorinated substitution on the nitrogen alters the activity of the molecule. β-chloropropyl or γ-chloropropyl can substitute for the β-chloroethyl radical. Methyl *bis*-β-bromine was slightly more active than its chlorine homologue and a fluorine congener was probably less active.

3. Environmental conditions have not been standardized in these experiments to permit an evaluation of differences in susceptibility of the different species.

4. In the range of 0.065–1.040 mM methyl-*bis*-(β-chloroethyl)-amine, retardation of second cleavage increases slowly with successive doublings of dose. Increasing the concentration beyond 1.040 mM rapidly decreases the percentage of eggs which divide.

5. The course of events in eggs exposed at the beginning of prophase, and later in the first and subsequent mitotic cycles points to a general slowing of mitosis. Any phase of mitosis can be blocked, depending upon the dose and time of exposure.

6. There is no synergism between urethan and methyl-*bis*-(β-chloroethyl)-amine when they are combined in threshold doses.

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STUDIES ON THE ACROSOME. I. REACTION TO EGG-WATER AND OTHER STIMULI[1]

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Our knowledge of spermatozoa and their role in the fertilization reaction has been acquired through studies approaching the subject from two widely different angles—"straight" morphological observation dealing with fixed specimens, such as the classical drawings of Retzius, or the electron microscope photographs of Bretschneider; and physiological studies centering around the fertilizin theory of F. R. Lillie and currently being pushed close to conclusion by the penetrating biochemical research of Tyler, Hartmann, Runnström and their co-workers.

One previous attempt to correlate the morphological and physiological aspects of sperm agglutination has been presented in the paper of Rötheli, Roth and Medem (1950), which deals with several species of freshwater fishes. The reaction in these forms, however, is an irreversible one, in which the spermatozoa become more and more firmly aggregated into increasingly dense clumps, and it seems difficult to distinguish possible normal changes in form due to agglutination from those accompanying the death of the spermatozoa.

In sea urchins, because of the small size and violent activity of spermatozoa in their normal state, work on living sperm has almost necessarily dealt with them in bulk suspensions; the vital staining study by Popa in 1927 is a rare example of an effort to examine living spermatozoa one at a time. A tendency in this direction is also to be found in Carter's 1931 paper, in which he stresses the fact that considerable physiological differences can be discerned between the gametes of a single induced shedding.

With the development of the phase contrast microscope, it has become possible to distinguish hitherto invisible details of active spermatozoa in a sufficiently normal state that observations can be made of their behavior under various conditions (Dan, 1950). Phase microscopy also provides a stepping-stone to the use of the electron microscope, by permitting a large degree of comparison between the living state and the electron photographs, and to a great extent obviating the uncertainty concerning the appearance of artifacts which would otherwise inevitably attach to a method involving observation *in vacuo*. All the points discussed in this paper have been checked in the living condition and in spermatozoa immediately after fixation in sea water suspension, and it can be stated with confidence that no qualitative difference was found among the three types of observation.

Materials and Methods

The spermatozoa photographed in this study belong to the two sea urchin species, *Pseudocentrotus depressus* and *Strongylocentrotus pulcherrimus*, although

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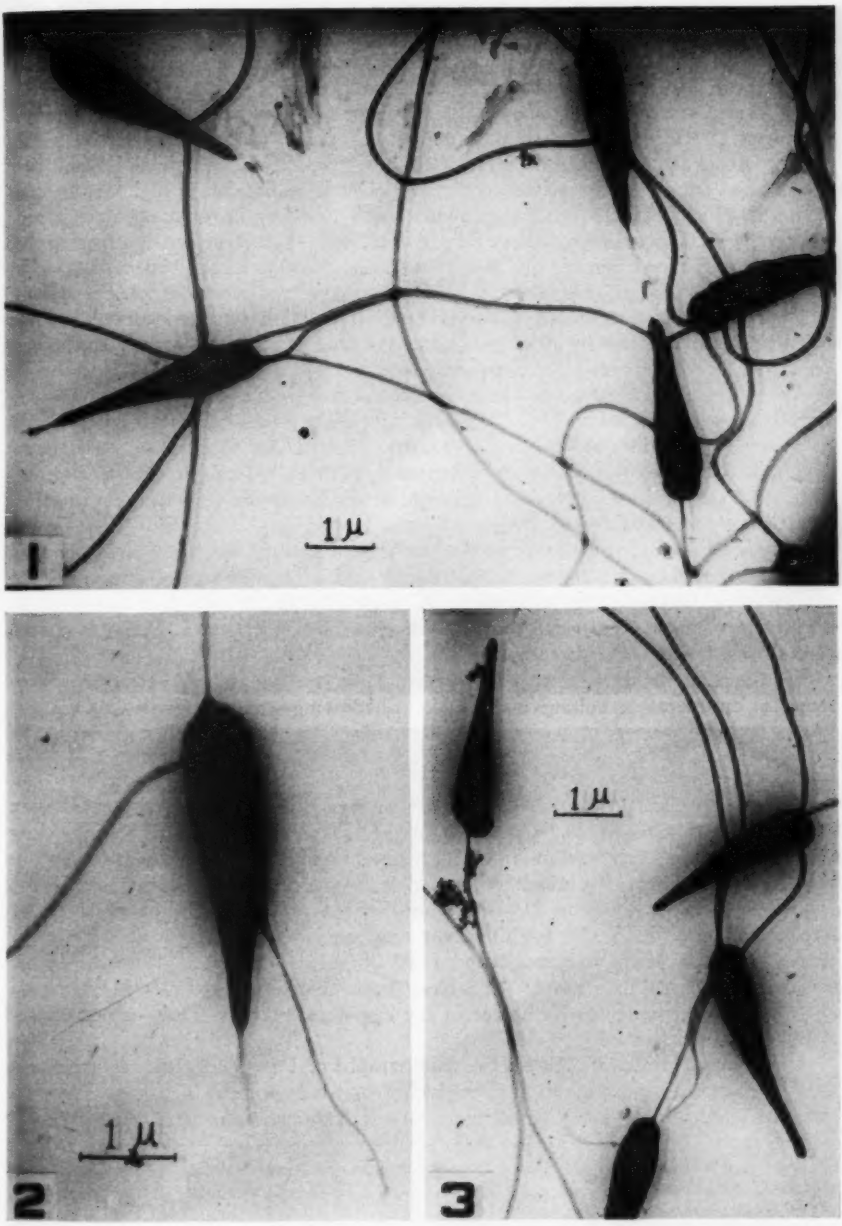

FIGURE 1. Spermatozoa of *Pseudocentrotus depressus*, fixed 30 seconds after the addition of egg-water.
FIGURE 2. Same as Figure 1.
FIGURE 3. Control in ordinary sea water.

phase contrast observation has shown that the phenomenon in question is generally characteristic of the other echinoids available at Misaki.

In every case, suspensions were made from dry sperm secured by removal of the testes with care to avoid contamination by sea water or body fluid; only freshly suspended spermatozoa were used. Egg-water was obtained by inducing shedding of eggs into filtered sea water (by electrical stimulation of intact females in the case of Pseudocentrotus (see Iwata, 1950), and by introducing a few drops of isotonic KCl into the cut test of Strongylocentrotus); the suspension was stirred occasionally during a minimum of two hours, and the titer of the supernatant jelly-sea water solution roughly determined by low-power microscopic observation of the intensity and duration of the agglutination reaction which occurred when a given amount of this egg-water was mixed with the standard sperm suspension (ca. 0.025 cc. dry sperm in 2 cc. sea water).

Irreversible aggregation of the spermatozoa was induced by suspending them directly in sea water of pH 9.2. For the first 30 seconds no heterogeneity could be detected in the distribution; from 40 seconds a tendency to clumping was apparent; by two minutes most of the spermatozoa were involved in aggregations approximately the size of those formed at the height of the reversible agglutination reaction. The suspension was fixed at this stage.

The spermatozoa were fixed by the addition of neutralized formalin to the suspensions; they were left for at least 24 hours in this solution before dilution of the sea water. The change to 5–7% formalin in distilled water was carried out by allowing the suspended spermatozoa to settle naturally, since it was found that even gentle centrifuging caused clumping.

Electron photographs were taken with a Hitachi standard type electron microscope, at an operating voltage of 50 KV. Shadowing was done with Cr_2O_3.

Results

In both species, the agglutination reaction occurs instantanously upon addition of egg-water to a sperm suspension; at the titer chosen as standard, the clumping of spermatozoa is at its maximum—*i.e.*, the greatest numbers of spermatozoa are involved in the clumps—at about 30 seconds after addition of the egg-water, and dispersal is complete within 2½–4 minutes. Figures 1 and 2 show spermatozoa fixed at the height of the agglutination reaction; Figure 3 is a photograph of spermatozoa similiarly suspended and fixed 30 seconds after the addition of the same amount of plain sea water. It is clear that a change has occurred in the region of the acrosome, under the influence of the egg-water; the probable nature of this change will be discussed below.

In Figures 4 and 5 are shown two photographs of Pseudocentrotus spermatozoa fixed 5 seconds after the addition of egg-water. It is important that in spermatozoa thus fixed immediately after reaction to the egg-water stimulus, the acrosome sub-

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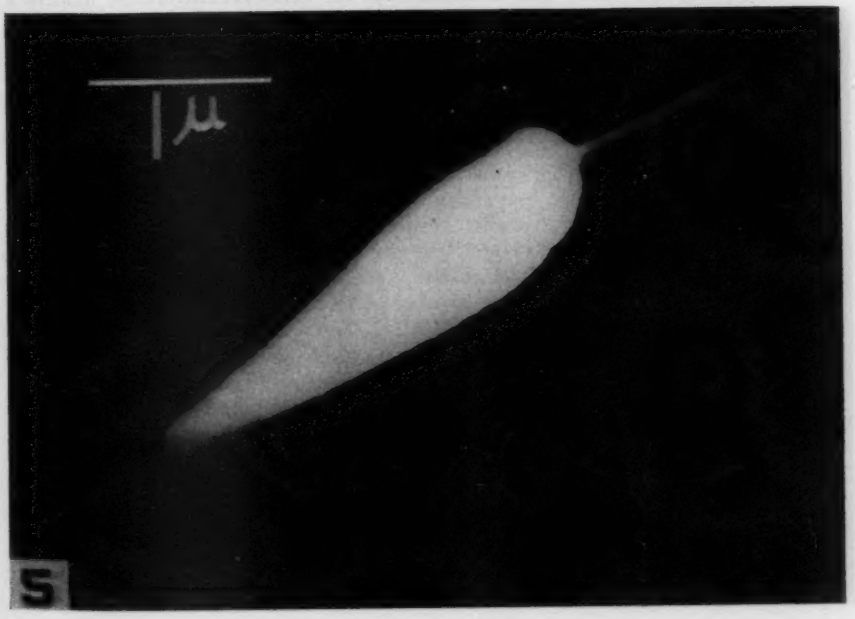
Figure 4. Pseudocentrotus spermatozoa fixed 5 seconds after addition of egg-water. The spermatozoan at the upper left has failed to react. The breakage between the head of the spermatozoan and the extruded acrosome substance is caused by drying on the collodion membrane. Shadowed with Cr_2O_3, $\tan^{-1} = \frac{1}{2}$.

Figure 5. Same as Figure 4, magnification 32,000 ×.

1μ
4


1μ
5

stance appears as a drop-like mass on the tip of the head. The break which almost invariably occurs between the tip of the sperm and the substance attached to it in such early fixation is interpreted as an artifact due to the contraction of the preparation on drying. Comparison of these photographs with Figures 6 and 7, of spermatozoa fixed at 2 minutes 35 seconds after reversal of agglutination, gives the impression that part of the acrosome substance disperses to a considerable extent, while a central core or fiber of some sort (which may be a separate structure that appears secondarily), possessing greater cohesiveness and not dispersing readily in sea water, remains attached to the former acrosomal region.

Figures 8 and 9 show photographs of Strongylocentrotus spermatozoa, fixed 25 seconds after addition of egg-water and plain sea water, respectively, to fresh suspensions. Figure 10, of spermatozoa fixed after two minutes in sea water at pH 9.2 indicates that morphologically the same sort of reaction is called forth by alkalinity as by egg-water.

Figures 11 through 15 are photographs of spermatozoa which were placed in living suspension (in plain sea water) directly onto a collodion membrane mounted for electron microscope observation, in order to test the possibility that the reaction of the acrosome may be induced by simple contact with solid objects, in the absence of egg jelly substance. The sperm were observed with low power of an ordinary microscope to ascertain that most of them were attached to the membrane by their tips and rotating. A drop of formalin-sea water was then added, at 105 seconds after suspension, and changed several times to remove unattached spermatozoa; after three hours fixation in formalin-sea water, the preparation was washed with dilute formalin and then distilled water before desiccation.

The photographs show that attachment to the collodion membrane induces a reaction essentially the same as that produced by egg-water. The acrosome substance is set free, appearing as an amorphous mass which fails to form a clear boundary in sea water and tends to spread on the collodion surface.

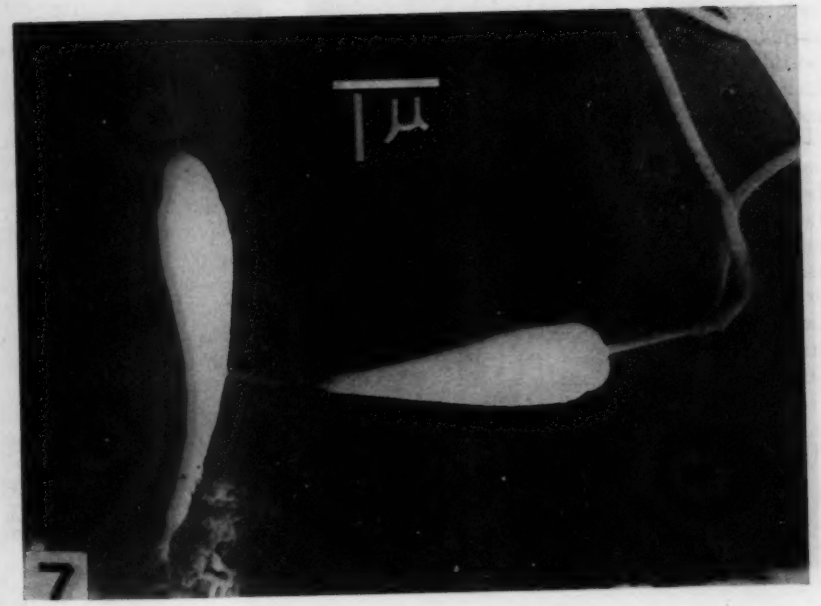
Discussion

Popa, after staining living *Arbacia punctulata* spermatozoa with Janus green, reports (p. 251), ". . . one gets the impression that the spermatozoa eliminates through the point of the head (where really there is an exceedingly minute opening) very small amounts of an extremely sticky substance." In egg-water (p. 256), "This appears as a small granule on the points of almost all spermatozoa. The adhesion of the spermatozoa to one another or to other objects is made by means of this granule."

With phase contrast oil immersion, it is easy to recognize Popa's "small granule" at the tip of the acrosome of unstained spermatozoa freshly suspended in sea water, of several sea urchin species. It is also just barely possible to make out a small mass of colorless, non-refringent substance attached to the tips of spermatozoa in egg-water (after reversal of agglutination, or immediately after addition of the egg-water if this is done under a cover-glass so that the spermatozoa become attached

Figures 6 and 7. Pseudocentrotus sperm fixed after reversal of agglutination (2 minutes 35 seconds after addition of egg-water).

1 μ
6


1 μ
7

to the glass surfaces instead of forming clumps). When such spermatozoa are freely suspended, the substance appears as a flabby, gelatinous "tongue" of uniform diameter (roughly 0.2 μ), which may reach a length of more than 1 μ and is always in vibratory, Brownian-like motion. That it is exceptionally sticky in normal sea water is attested to by the fact that once this substance has come into contact with any surface, it is never pulled loose even by the most vigorous movements of the spermatozoan. A few cases have been observed in which active spermatozoa have broken away, leaving at least part of the "tethering" substance on the glass surface, but it is much more common to find them permanently attached by the highly pliable strand which obviously possesses considerable cohesiveness.

As for Popa's "minute opening," however, it is probable that what he was seeing was rather a denser portion at the extreme tip of the acrosome, which appears darker than the more proximal part with phase contrast.

Before this study reached the stage of electron microscopy, it seemed fairly apparent that a gelatinous substance was extruded from the tip of the acrosome in response to the stimulus of egg-water, alkaline sea water and contact with solid surfaces. However, careful study of all the electron photographs seems to indicate rather that the anterior part of the acrosome undergoes a drastic change within one or two seconds after such stimulation. This is most clearly shown in Figure 4, in which the one spermatozoan which has failed to respond immediately to the egg-water stimulus is long and tapering at the apex, while the two others which show the typical reaction are truncated.

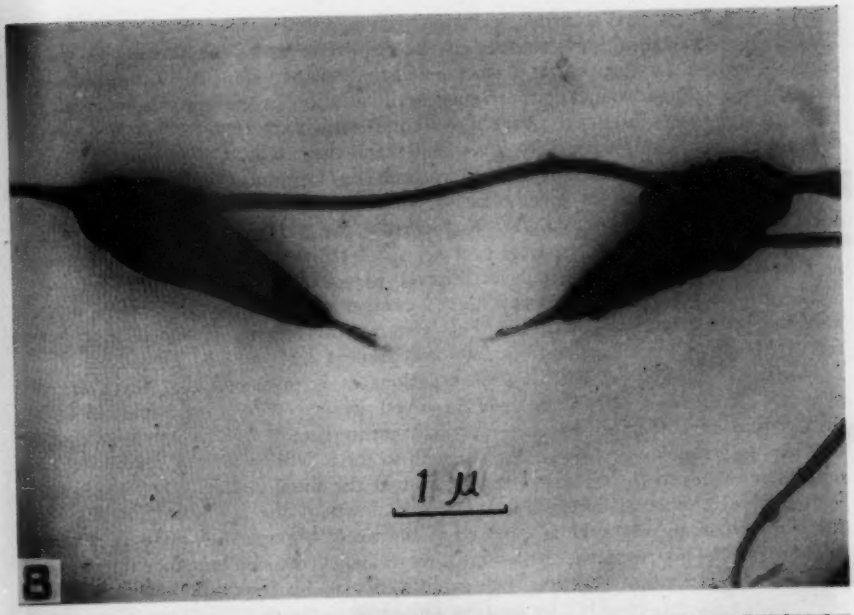
It is proposed that the condition of the acrosomal region in sperm which have so reacted (*cf.* Fig. 5) can best be explained by imagining that the plasma membrane investing the tip of the acrosome undergoes local autolysis, leaving the underlying substance exposed. If this substance is, or includes, an *egg-membrane* lysin such as that isolated by Tyler (1939) and Krauss (1950a, 1950b), or an *egg-surface* lysin like Androgamone III of Runnström *et al.* (1944, 1945, 1946), the postulated breakdown of the acrosome membrane would leave the lysin free to attack the vitelline membrane of the egg as soon as the spermatozoan carried it into contact with the egg surface.

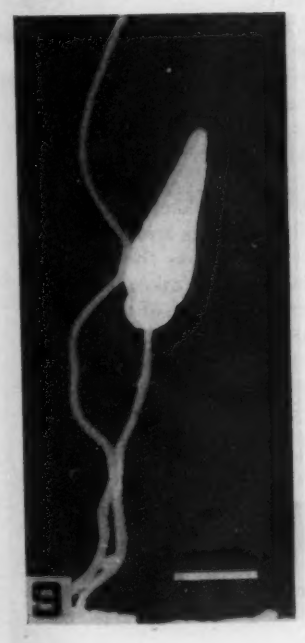
The two widely differing lytic substances identified by these two groups of workers are obtained from frozen-thawed or frozen-dried sperm; there has so far been presented no unequivocal evidence as to their ready availability or exact location in the living spermatozoan, although Tyler (1949) has published electron micrographs of keyhole limpet spermatozoa showing that extraction with alkaline sea water (pH 9), which yields a solution of the egg membrane lysin, causes a more or less drastic breakdown of the acrosome. Pending tests of its chemical properties and lytic activity, it can only be offered as a surmise that the acrosome substance under discussion in this paper contains either or both of the lysins identified by Tyler and Runnström.

FIGURE 8. Strongylocentrotus spermatozoa fixed 30 seconds after agglutination with egg-water.

FIGURE 9. Strongylocentrotus sperm fixed 30 seconds after addition of plain sea water to suspension (control).

FIGURE 10. Strongylocentrotus sperm fixed after 2 minutes in sea water of pH 9.2.


1 μ
8


9

1μ
10

In support of that surmise, certain circumstantial evidence can be adduced. The time relationships satisfactorily fit the requirements of the process as it must occur in normal fertilization, if it is assumed that the acrosome membrane is stimulated by the jelly substance, either intact or in solution in the immediate vicinity of the egg, and reacts during the very few seconds which are required for the spermatozoan to pass through the jelly layer and reach the vitelline membrane.

The fact that this reaction of the acrosome is also induced by hyperalkalinity in the absence of the species specific factor contained in the egg jelly would account for the long-known empirical fact that raising the pH of the medium facilitates sperm penetration in inter-species crosses, self-fertilization in self-sterile hermaphrodites, and other cases in which fertilization is blocked in normal sea water.

Furthermore, the observed reaction to contact with solid objects offers a fairly satisfactory explanation for the fact that removal of virtually all the jelly from unfertilized eggs by acid extraction does not prevent fertilization.

On the basis of these various observations, it is proposed that there are two separate phenomena simultaneously involved in egg-water agglutination of sea urchin sperm: a specific reaction (fertilizin-antifertilizin) which orients the spermatozoa into regular groups and holds them so for a definite period; and a response of the acrosome to the chemical stimulation of the dissolved jelly, which is manifested by an almost instantaneous local breakdown of the acrosome membrane so that the acrosome substance is exposed at the tip of the sperm head as a relatively labile mass. This response of the acrosome is believed to be less specific in nature than the agglutination reaction, being induced also by hyperalkalinity and (possibly somewhat less readily) by contact with solid surfaces.

Applying this experimental conclusion to natural fertilization, it is suggested that as the spermatozoan actively swims through the loose network of the jelly layer, it responds to the chemical stimulation of the jelly substance by a breakdown of the membrane covering the front part of the acrosome, so that by the time the sperm reaches the egg surface, a few seconds later, it carries at its tip a mass of freshly exposed lysin with which it effects penetration of the vitelline membrane as the first step in the fertilization process.

In a discussion of the properties of fertilizin, Tyler (1949) makes the following statement (p. 203): ". . . experiments show that fertilizin acts as an aid to fertilization, but only when it is present on the surface of the egg, as the gelatinous coat. . . . Evidently when the interaction of the egg and fertilizin takes place away from the egg and is completed before the sperm reaches the surface of the egg, the sperm are then unable to combine with the egg." Assuming that the sea water-labile portion of the acrosome substance is a vitelline membrane lysin, its demonstrated dispersal within less than 30 seconds after contact with egg-water (fertilizin)

FIGURES 11–13. Spermatozoa of *S. pulcherrimus;* freshly suspended sperm fixed on collodion membrane, showing various degrees of acrosome reaction.

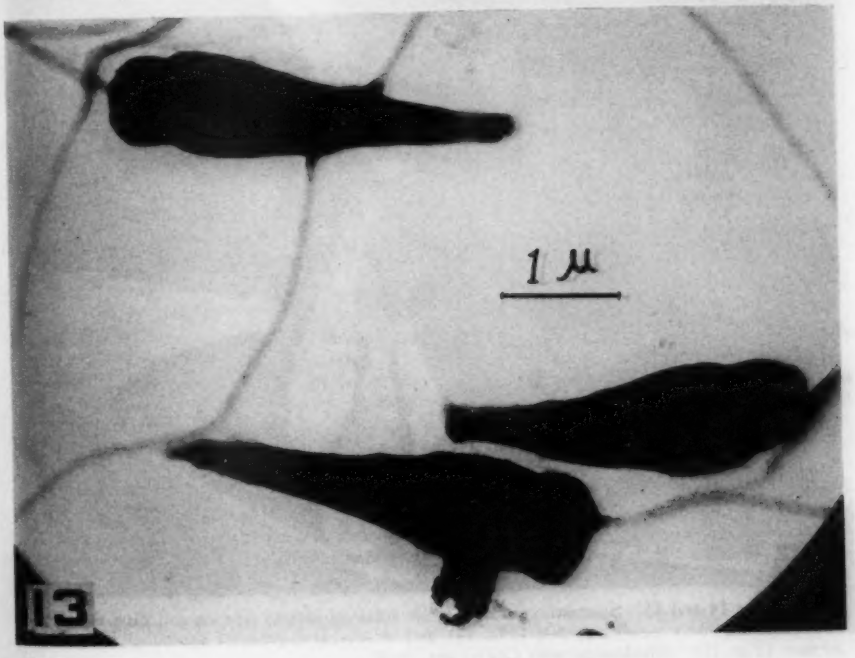
FIGURE 11. Acrosome partially reacting to stimulus of its own tail.

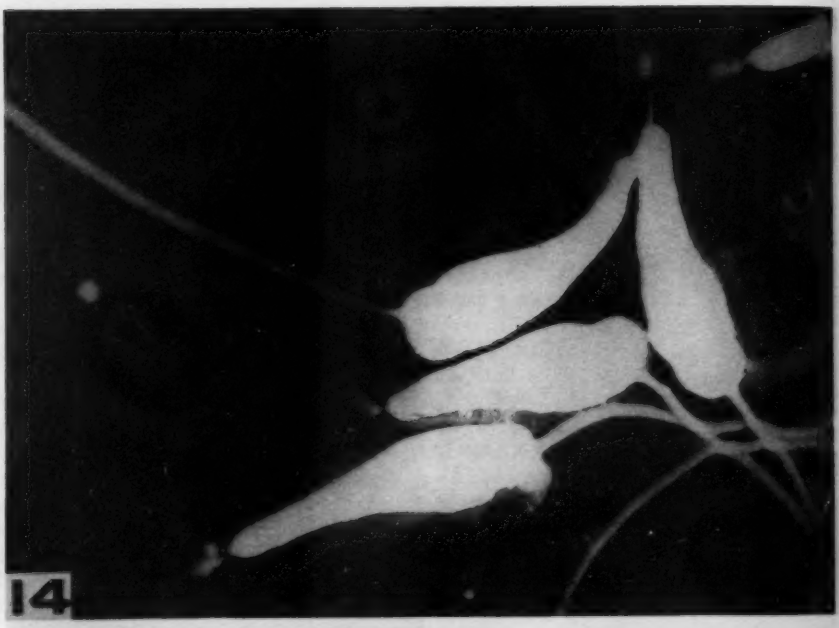
FIGURE 12. Acrosome of sperm at right completely reacted to contact with collodion membrane.

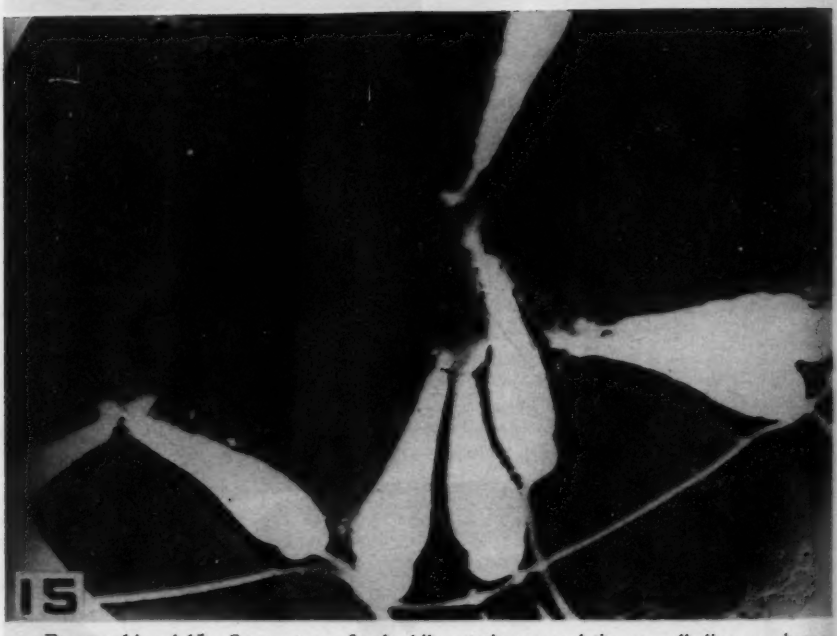
FIGURE 13. These three spermatozoa were rotating about their tips on the collodion membrane when fixed. (This photograph was intentionally under-exposed to show differences in density of acrosome region. Fully exposed print shows acrosome substance spread on collodion membrane around points of attachment, similar to condition in Figure 15.)

1 μ
11

1 μ
12


1 μ
13





FIGURES 14 and 15. Spermatozoa fixed while rotating around tips on collodion membrane. Acrosome substance newly exposed (Fig. 14) and in several degrees of spreading on collodion surface (Fig. 15). Shadowing with Cr_2O_3; $\tan^{-1} = \frac{1}{8}$.

would seem to furnish an adequate explanation for the subsequent failure of the spermatozoan to effect penetration of the egg.

Krauss (1950a) has worked out in great detail the conditions under which egg-membrane lysin is obtainable from spermatozoa of the keyhole limpet. One of his most striking cases, that of increased pH, coincides with the result reported in this paper, and there is nothing in his other observations which is contrary to the suggestions made here. Coordination of the experimental conditions might very well bring out an interesting positive agreement between the two sets of results.

The writer wishes to express sincerest gratitude to Prof. A. Takamiya, of Tokyo Institute of Technology, for his enthusiastic encouragement and kindness in arranging for the use of the Institute's electron microscope; and to Mr. H. Akabori, of the Institute, who has cooperated most generously in preparing and photographing the material. The writer's thanks are also due in large measure to the Director and Staff of the Misaki Marine Biological Station for their unfailing courtesy in sharing the Station's facilities; and finally to Drs. C. B. Metz, D. Mazia and K. Dan, for their exceedingly helpful criticism of the manuscript.

Summary

1. Spermatozoa of two sea urchin species were fixed with formalin in sea water, egg-water and sea water of pH 9.2, and observations of fixed specimens made with phase contrast and electron microscopes were compared with phase contrast observation in the living state.

2. In response to egg-water, a reaction is called forth which is interpreted as a breakdown of the acrosomal membrane, by which a labile mass of substance is exposed at the tip of the spermatozoan head.

3. This same reaction is induced by exposure to sea water of pH 9.2, and by contact with solid surfaces.

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THE INFLUENCE OF TEMPERATURE ON SYNAPSIS IN HYBRID SALIVARY GLANDS [1]

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The giant chromosomes of Diptera are the most favorable material for studies of the extent and nature of synaptic association. The mitotic and meiotic chromosomes in this group are so small that they hardly offer a clear picture of the details of their conjugation during prophase or interphase. It has become customary, therefore, to rely on salivary glands for studies of synaptic association in structural hybrids of Drosophila, although it is not known whether the synapsis of giant chromosomes reflects with any degree of accuracy the mode of association of homologues in meiosis or mitosis. While the conjugation of homologues is believed to be a prerequisite for the occurrence of crossing-over, the observed variations in cross-over frequency and their relationship to the phenomenon of synapsis are not understood. It seems desirable, therefore, to determine whether synaptic association varies with environmental factors, which are known to influence the frequency of crossing-over. Beginning with Plough (1917), much attention has been paid to the effect of temperature on crossing-over. It was for this reason that temperature was chosen as the varying factor in the present study.

The question of variation in synapsis, apart from its possible bearing on crossing-over, gains additional interest in the light of the "Structural Theory" of the position effect mechanism (Ephrussi and Sutton, 1944; Gersh and Ephrussi, 1946).

Variation in the extent of synapsis with age and temperature was observed in salivary glands of Chironomus hybrids (Goldschmidt, 1947). Synapsis was found to decrease with rising age of the larvae. In larvae grown at high (30° C.) and low (13° C.) temperatures, there was less synapsis than in larvae reared at moderate temperatures (20–25° C.). While salivary glands of Chironomus offer material for smears during an extended prepupal period, the suitable stage for smearing in Drosophila is of very short duration. This precise definition of the physiological age suitable for smearing should tend to minimize any possible age effect on synapsis in preparations obtained from this organism and should render them a suitable object for the study of the temperature effect.

Materials and Methods

The effect of temperature on synapsis was examined in two structural hybrids in the genus Drosophila. As an instance of a small inversion within a species, the

[1] This work was carried out in the Zoology Department, University of California, Berkeley, during the tenure of the Mary E. Woolley International Fellowship of the American Association of University Women, awarded by the International Federation of University Women.

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Arrowhead inversion in Chromosome III of *D. pseudoobscura* was chosen. For a large inversion in a species hybrid, the condition of Chromosome III R in the *D. melanogaster* × *simulans* hybrid was studied.

For the *D. pseudoobscura* crosses, 10 females and 10 males were mated in half-pint bottles and allowed to oviposit for three days at room temperature. The parents were then removed and the bottles transferred to constant temperature chambers of 13, 17.5, 20, 25 and 28° C. (this being the upper limit for larval development in this species).

The *D. melanogaster* × *simulans* cross was obtained by the method described by Uphoff (1949), 10 *melanogaster* females and 15 *simulans* males being mated (without etherizing) in "creamers." If the cross was found to be fertile, the parents were removed to half-pint bottles and allowed to oviposit for two days, at room temperature. After removal of the parents, the bottles were kept at 13, 17.5, 20, 25, 29 and 31° C. Although the food employed was molasses-cornmeal-agar enriched with yeast, "yeasting" of the bottles at the time of appearance of second instar larvae was found indispensable for obtaining good preparations of salivary chromosomes.

The glands were dissected, at room temperature, in 45% acetic acid, stained in aceto-carmine and made permanent according to a method of Dr. A. M. Hannah, by transferring through alcohol and xylol into Canada Balsam, without removing the cover-slip, whenever possible.

The preparations examined for the *pseudoobscura* cross include male and female offspring of reciprocal matings between Standard and Arrowhead. The *melanogaster* × *simulans* preparations were all from female larvae of the cross employing *melanogaster* as the female parent.

The following arbitrary system of recording asynapsis was adopted: *D. pseudoobscura*. The loop extends from band 70B to 76B in Chromosome III (Dobzhansky, 1944).

	Asynaptic units
Loop completely unsynapsed	3
Loop synapsed except for sections 70B–D and/or 76A–B	1
Loop synapsed less than this	2
Complete asynapsis in section before loop (63A–70A)	1
Any degree of asynapsis in section before loop	½
Complete asynapsis in section after loop (76C–81D)	1
Any degree of asynapsis in section after loop	½

D. melanogaster × *simulans*. The loop (figured by Pätau, 1936) extends from band 84F to 93E.

	Asynaptic units
Loop completely unsynapsed	4
Loop three-quarters unsynapsed	3
Loop half unsynapsed	2
Loop one-quarter unsynapsed	1
Loop less than one-quarter unsynapsed	½
Section before loop (81F–84F) completely unsynapsed	1
Section before loop partly unsynapsed	½
Section after loop (93F–100F) completely unsynapsed	3
Section after loop two-thirds unsynapsed	2
Section after loop one-third unsynapsed	1
Section after loop less than one-third unsynapsed	½

In order to avoid unconscious selection, the labels of all preparations were covered with non-transparent paper before scoring. Twenty nuclei were scored in each preparation of the *pseudoobscura* cross and ten nuclei in each of the *melanogaster* × *simulans* crosses.

Results

a) *Arrowhead* × *Standard inversion*

The results are presented in Table I and Figure 1. There was more asynapsis at 13° C. on one hand and at 28° C. on the other than at intermediate temperatures (17.5–25° C). Although the standard errors are large, the difference between 13° C. and 17.5° C. and the difference between 25° C. and 28° C. are significant. The slight rise in asynapsis between 17.5° C. and 20° C. and the decline from 20° C. to 25° C. are not significant. It may be concluded that synapsis in this inversion is comparatively most complete at intermediate temperatures. The large standard errors reflect, of course, the considerable differences existing between different individuals grown at the same temperature. Moreover, as is known to every worker with salivary gland chromosomes, the variation between nuclei of the same gland is also pronounced. Nevertheless, a cursory inspection of a preparation was usually sufficient to determine whether the larva had grown at an extreme or at an intermediate temperature.

Table I

Asynaptic units in Arrowhead-Standard inversion

Temp. °C.	No. of larvae	Mean asynaptic units	Standard error	t	p
13	15	30.2	±7.3		
				3.2	0.01–0.001
17.5	14	22.5	±5.8		
				0.7	0.6 –0.5
20	13	23.7	±3.2		
				1.3	0.3 –0.2
25	14	21.3	±6.0		
				4.3	<0.001
28	13	30.0	±4.3		

b) *Melanogaster* × *simulans inversion*

The results are summarized in Table II and Figure 1. Synapsis is most complete at 13° C. There is less pairing at 17.5° C. Asynapsis falls off from 17.5° C. to 25° C. and then rises again until 31° C. The differences between 13° C. and 17.5° C., 17.5° C. and 25° C. and between 25° C. and 31° C. are significant.

It should be mentioned that the preparations of the 13° C. series could usually be assigned to this group without reading the label. Although they do not show significantly more synapsis than those of the 25° C. group, their chromosomes are peculiar, because the fusion of the homologues in synapsed regions is usually less intimate than in any other series. Since under the present system of scoring any mode of contact between homologues was recorded as "synapsis," the particularly loose synapsis of the 13° C. group is not expressed in Table II.

TABLE II

Asynaptic units in melanogaster × simulans inversion

Temp. °C.	No. of larvae	Mean asynaptic units	Standard error	t	p
13	22	15.4	±5.2		
				3.3	0.01–0.001
17.5	15	22.6	±8.0		
				0.7	0.5 –0.4
20	13	20.5	±7.2		
				1.4	0.2 –0.1
25	17	17.3	±5.3		
				1.4	0.2 –0.1
29	17	19.6	±4.5		
				2.4	0.05 –0.02
31	10	23.8	±4.3		

Significance of difference between:

Temperatures ° C.		
13 and 25	1.1	0.3 –0.2
17.5 and 25	2.2	0.05–0.02
25 and 31	3.3	0.01–0.001
13 and 31	4.5	<0.001

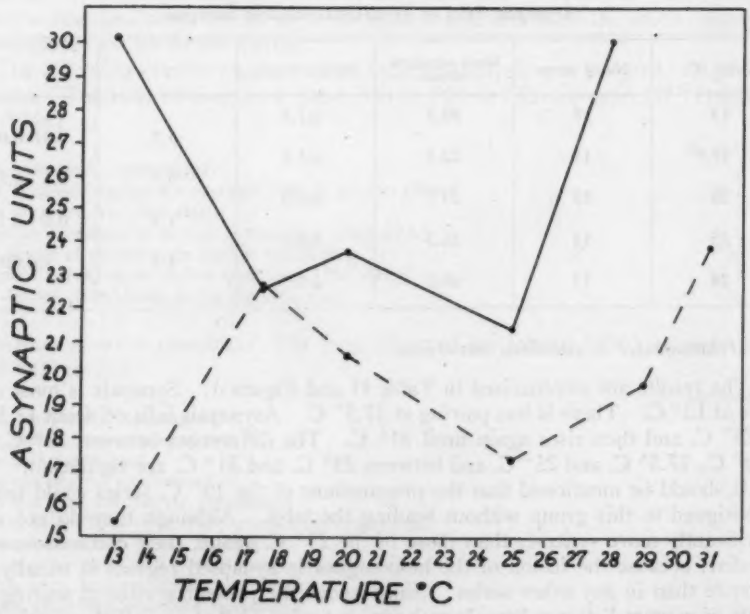

FIGURE 1. Variation with temperature of asynaptic units. Heavy line: Arrowhead × Standard inversion in *D. pseudoobscura*. Broken line: *D. melanogaster* × *simulans* hybrids.

Discussion

Since all the results are based on smears, it might be doubted whether they reflect the true association of homologues in intact nuclei. Hinton (1945) showed that terminal associations are not easily broken, even when stretched with a micromanipulator. This observation makes it unlikely that truly synapsed regions should be separated by the pressure exerted in smearing.

In the two graphs presented in Figure 1, the common features are the minimum of asynapsis at moderate temperatures and the rises on either side of the intermediate range. The most outstanding difference lies in the presence of a second minimum of asynapsis in the *melanogaster* × *simulans* inversion and its absence in the Arrowhead × Standard cross. It has been mentioned that at 13° C., synapsis in the *melanogaster* × *simulans* hybrid is extensive, but not intimate. This phenomenon renders a strict comparison of the two graphs at the 13° C. level impossible.

The presence of a minimum of asynapsis at moderate temperatures in the two rearrangements investigated confirms the findings in Chironomus (Goldschmidt, 1947). However, in view of the considerable differences existing, especially between the *melanogaster* × *simulans* cross and the other two, the combined data do not justify the assumption that we are dealing with a phenomenon universal in structural hybrids. It should be remembered, moreover, that the results obtained with structural hybrids may not have any direct bearing on the process of synapsis in structurally homozygous individuals.

Caution in evaluating the present data seems especially indicated in consideration of the complex evidence regarding the influence of temperature on crossing-over and on chiasma frequency. Plough (1917, 1921) found a minimum of crossing-over at moderate temperatures in the autosomes of *D. melanogaster*, but could not confirm this effect for the X-chromosome in regions distant from the centromere. Stern (1926) found a temperature effect in the X-chromosome near the spindle attachment (from garnet to bobbed). Clark (1943) did not detect any influence of temperature on crossing-over in Habrobracon. White (1934) obtained a curve similar to that of Plough for chiasma frequency in Stenobothrus, but his graphs for Locusta and Schistocerca differ markedly from this pattern. The situation is further complicated by the data of Stern and Rentschler (1936) on somatic crossing-over in the X-chromosome of *D. melanogaster*. This is high at both 17 and 25° C. and low at 30° C.

Much significance has been attributed to the resemblance of Plough's curve to the variation of plasma viscosity with temperature (Heilbrunn, 1928; Frey-Wyssling, 1948). The temperature-viscosity curves of some organisms exhibit several maxima and minima within the biological temperature range. If the absence of a simple linear relationship to temperature may indeed be considered as a common characteristic, this is certainly shared by the graphs presented here. The minimum of crossing-over at intermediate temperatures is paralleled by a minimum of asynapsis in a similar temperature range: a paradoxical situation in view of the fact that chromosomes presumably do not cross-over unless they are previously paired. This paradox might resolve itself if local repulsions between paired homologues proved to be involved in the mechanism of crossing-over.

The influence of temperature on various position effects, previously established by Gowen and Gay (1933, 1934) and various later workers, was studied more recently by Hinton (1949) and Gersh (1949). Their results indicate that with a number of position effects, extreme temperatures produce either smaller or larger effects than moderate temperatures. Since synapsis in some structural hybrids is similarly affected by temperature, it seems possible that some position effects of the variegated type may indeed be connected with synapsis variations, setting up different stresses in the chains of gene proteins.

Summary

1. The effect of temperature on synapsis was studied in salivary glands of *Drosophila pseudoobscura* heterozygous for the Arrowhead inversion, and of *D. melanogaster* × *simulans* hybrids.

2. In the *pseudoobscura* larvae, significantly less asynapsis was observed at 17.5, 20 and 25° C. than at extreme temperatures (13 and 28° C.).

3. In the *melanogaster* × *simulans* hybrids, synapsis was most complete at 13 and at 25° C., and there were two significant peaks of asynapsis at 17.5 and at 31° C.

4. The common feature of the two distributions is the minimum of asynapsis at moderate (20 and 25° C.) temperatures. The possible relationships of this result with the known temperature effects on crossing over and on the position effect are discussed.

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STUDIES ON THE FLAGELLA OF ALGAE. I. GENERAL OBSERVATIONS ON CHLAMYDOMONAS MOEWUSII GERLOFF [1]

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Flagella are the protist prototypes of muscles, presenting perhaps the simplest systems in which we may investigate the processes which knit biochemical structure with the mechanics of motility (*cf.* Dellinger, 1909). The literature on their structure and mode of action in the flagellates has been reviewed by Brown (1945), Owen (1949) and Pitelka (1949). Lowndes (1941) has carried out a series of observations on motility of these organisms, using high-speed cinematography. Tactic behavior patterns in *Chlamydomonas* spp. have been described by Desroche (1912), Kuwada (1916) and Cadoret (1949).

Chlamydomonas moewusii (Chlorophyceae, Volvocales) is a biflagellate unicellular alga which has proved to be a suitable organism for studies of flagellar activity. The presence of chlorophyll enables the cells to multiply in media in which CO_2 is the only source of carbon, and under suitable conditions of illumination their numbers may be quadrupled daily. If their movement is arrested, the flagella may be seen in unstained living cells under high magnifications; they are found not only to be concerned with motility, but also to be involved in a remarkable manner with the processes of mating. Moreover, this species of *Chlamydomonas* is haploid and heterothallic, rendering it suitable for genetic investigation; so that mutants in which motility has suffered some inherent impairment can be crossed and their progeny analyzed for possible recombination, etc.

This paper is to be concerned mainly with general aspects of the flagella of *Chlamydomonas moewusii*, visible under the light microscope in unstained preparations, or in preparations stained with gentian violet following osmic fixation, according to the technique given by Couch (1941). Studies on their fine structure, as revealed by the electron microscope, and on certain physiological aspects of motility and mating are in preparation.

1. Morphology

The two flagella of normal cells arise in the region of a chromophilic body at the anterior extremity of the protoplast, and extend to the exterior through separate perforations of the wall, on either side of the papilla. These pores, in empty walls from which the contents have escaped, are readily visible under the phase-contrast microscope or in electron microscope preparations.

[1] This work was carried out during tenure of a Sheffield–H. D. Hooker Fellowship at the Graduate School, Yale University, and with the assistance of a Lucretia Crocker Fellowship at the Marine Biological Laboratory, Woods Hole, Massachusetts.

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The flagella themselves are approximately 12–24 μ in length. They vary considerably in older cultures, in which cells with short flagella 2-3 μ in length, and some totally devoid of flagella, may be found on the bottom of the culture vessel. Short flagella may vibrate actively, but the cells are unable to swim. Normal, long flagella are typically geniculate, with a slight bend about 3–4 μ from the point of attachment. Under no conditions has a mastigonema or whiplash been seen, though such a structure has been described for many other species of *Chlamydomonas* (*e.g.*, by Deflandre, 1934; Owen, 1949). In suitably fixed material of *C. moewusii* examined under the electron microscope, the flagella can be seen to terminate in a short mucro, about 0.3–0.4 μ in length.

Preceding vegetative division and shortly before the fusion of gametes, the flagella are withdrawn, the process taking about 30 minutes for completion. In unfavorable conditions, usually leading to death of the cells, the flagella may be shed; almost any irritant (*e.g.*, 10^{-3} *M* acetic acid or 5×10^{-3} *M* ammonia) will produce this effect.

In other conditions, the flagella may undergo a pathological transformation into vesicles flanking the papilla; this transition, which occupies less than a minute, may be observed microscopically. Desroche (1912) found that this change could be induced in *Chlamydomonas obtusa* by subjecting the cells to temperatures close to 40° C. for some minutes. The phenomenon was observed also in *Polytoma* by Francé (1894), Dangeard (1901) and Korschikov (1923), and is discussed by Brown (1945).

Flagella are formed by cells growing on the surface of 1% agar media, and can readily be seen when young cultures are examined *in situ*. On flooding, in darkness or light, the cells are immediately capable of swimming. When the pH of the medium, liquid or agar, falls below 6.0, flagellated cells are replaced during growth by palmella.

2. *Swimming*

Normal flagellar activity has been described for many cells of this type (*e.g.*, Ulehla, 1911). Briefly, it resembles in essentials the movements involved in "breast-stroke" swimming, and is accompanied by a rotation of the whole organism. At 20° C. the rate of flagellar action lies between 10.5 and 12.2 beats per second. (These values were obtained by a simple stroboscopic device in conjunction with dark-field illumination (Richie, 1950) and phase-contrast objectives. Lowndes (1941), using a cinematographic method, observed 8 beats per second in morbid specimens of an unidentified species of *Chlamydomonas*.) Since the position most readily "fixed" by the stroboscope is that in which the flagella extend outward at the sides of the cell (this, too, agrees with the observations of Ulehla, 1911), it is apparent that propulsion is chiefly effected by the downstroke, during which the cell is drawn forward while the ends of the flagella move comparatively little.

Cells of old cultures tend to exhibit a disturbed synchrony of the flagellar pair. One flagellum may cease to beat for a period, or may beat irregularly, with the result that progress is erratic, the cells frequently tumbling and turning in small circles or spinning about the inactive flagellum.

In old cultures one frequently may observe a temporary reversal of the normal direction of progress. For periods of 1–2 seconds, a cell may proceed backwards,

without rotating, with its flagella extended parallel from the papilla and vibrating rapidly at the tips. This mode of swimming, characteristic of the related genus *Scourfieldia*, has been observed as sporadic behavior in other species of *Chlamydomonas* (Ulehla, 1911; Oltmanns, 1922).

3. Creeping

In examining related organisms (*Polytoma* and *Chlamydomonas* spp.), Francé (1894) and Ulehla (1911) made an interesting observation which has apparently received no attention from later authors. They independently reported that the flagella were capable of creeping along a solid substratum. This has been substantiated by the present author, who first observed it in cells of the paralyzed mutant, M. 236, of *C. moewusii* (Lewin, 1952) in which the flagella do not beat, but extend from the body of the cell. In microscope mounts, the tip of the flagellum can be seen to execute a serpentine motion along the surface of slide or cover-slip; the movement appears autonomous, but ceases as soon as a flagellum becomes detached from its cell. The rate of movement at room temperatures approximates 2 μ per second. If one flagellum is grounded and the other free (or missing), the cell is drawn along thereby; if both flagella extend forward, more or less parallel, the same movement is achieved. However, more usually the flagella are extended in opposite directions, in which case they stretch apart as far as possible, while the cell remains stationary, with its papilla pressed against the substratum. This accounts for the frequency with which one encounters *Chlamydomonas* cells end-on in microscopical preparations, as figured, for example by Gerloff (1940, Figs. 1 and 2).

Two possible mechanisms might be conceived to account for this movement:

1. The action of secondary cilia might be postulated.
2. Peristaltic waves or undulations may be propagated basipetally from the tips.

The first possibility is in this case immediately excluded since the surface of the flagella is seen under electron microscopy to be quite devoid of lateral appendages. This is in agreement with all other observations on the flagella of members of the Volvocales (*cf.* Brown, 1945).

The possibility then remains that the creeping of the flagella is attributable to pulses travelling down from the tip—which unfortunately has not been visibly confirmed, since the flagella are considerably less than 1 μ in width. In fact, Ulehla (1911) described in non-beating flagella of *Gonium* the uninterrupted passage of two or more spiral waves within a flagellar sheath. Their movement, however, was acropetal.

4. Mutants

Mutants in which the flagella are lacking altogether (though the basal chromophilic body remains), and others in which the swimmming (but not the creeping) faculty is lost, have been obtained following ultra-violet irradiation and are described elsewhere (Lewin, 1952).

5. Mating behaviour

a. Clumping. The species is regularly heterothallic and isogamous: complementary mating types have been arbitrarily designated as "plus" and "minus." When suspensions containing sexually active plus and minus cells are mixed, the phenomenon of clumping occurs. It can be observed that in this species only the smaller, younger cells take part in this sexual response, while the larger cells are capable only of vegetative reproduction. The young cells may be said to be facultatively gametic, and it is only when in this state that they are capable of aggregating into clumps. Clumping is attributable to a specific "stickiness" of the gamete flagella, by which cells of opposite mating-type become attached to one another (Gerloff, 1940). The aggregation appears to follow random contacts; in dilute suspensions the cells tend to associate in twos and threes, while in crowded conditions clumps of 50 or more cells may form within 1–2 minutes of mixing, and may continue to grow for several minutes.

b. Pairing. The specific stickiness of the flagella of gametes during pairing can be compared, perhaps, to an antigen-antibody association between proteins, resulting initially in adherence of the flagella at any point of contact. Furthermore, a *polar* orientation of the pairing flagella is induced, so that they come to lie parallel with one another while extending in the same direction relative to their respective cells. In other words, they orient distal to distal end, proximal to proximal.

By some unascertained means, cells within a clump succeed in coupling so that both flagella of the partners are paired; probably this takes place during the random flailing of these organelles. They do not become entangled (as has been stated in the literature for other species—probably incorrectly), but adhere for a large part of their length. According to the position of the original points of adherence, the relation of the pairing cells will be at first more or less skew (*i.e.*, not coaxial), and now a further organizing mechanism comes into play. As has already been mentioned, the individual flagella have an ability to creep over surfaces. This faculty now enables pairing cells to adjust their spatial relations, by sliding their flagella along those of the partner, until their respective papillae are in apposition. Such sliding adjustment may perhaps expain the polar association of flagella, mentioned above. Flagella may possibly pair at first in an antipolar (*i.e.*, distal-to-proximal) fashion, but disengage shortly afterwards at their free ends. This, however, has not been observed.

The cells become appressed, papilla to papilla, and about 5 minutes after the initiation of mating a protoplasmic bridge is formed between their anterior ends (*cf.* Korschikov, 1927). Another 5 minutes later, the flagella suddenly lose their stickiness and, with it, any tendency for the pair to remain with others in a clump. The four flagella of the copulants disengage to extend freely in the medium, and the pair soon swims away.

c. Behavior of pairs. During the changes related in the previous section, the cells have been engaged in much to-and-fro movement within a struggling mass, and this apparently disorganized activity persists for a minute or two in newly-united pairs. Its place is very soon taken, however, by a regular, uni-directional mode of swimming, in which the pair behaves as a single entity and the conflicting activity of the two sets of flagella is no longer, or only sporadically, apparent. In some way, therefore, the motor apparatus of the two copulants has become ad-

justed so that directed locomotion (*e.g.*, positive phototaxis) is once more feasible. The natural advantage of such a coordinating device is clear.

Close examination of such pairs reveals two notable features: (1) Whenever a slight discrepancy in size, form or color enables one to distinguish the partners one from the other, it can be observed that the relationship of the two cells is constant. One cell retains its anterior position, while its mate remains in the rear, throughout the swimming period of the pair. (2) The flagella of the posterior partner are wholly responsible for the motility of the pair, beating in apparently normal fashion. Those of the anterior cell, their plane now perpendicular to that of the driving cell (as observed in *Phyllocardium* by Korschikov, 1927), extend along the body of the partner, and rarely exhibit more than a slight twitching of the tips.

To establish whether the differentiation in flagellar activity is genetically inherent in the respective mating-types, the cells were labelled in various ways, so that they could be distinguished by visible microscopic characters. Lerche showed how this could be done in *Dunaliella*, by mixing starved gametes of one mating-type, in which a red carotinoid had accumulated, with well-nourished green cells of the opposite type (Lerche, 1937, Plate 6).

In *C. moewusii* it has been possible to carry out similar observations, by mating starved cells with normal cells. Alternatively, the gametes have been rendered distinguishable by intravital staining (*e.g.*, with dilute neutral red), or by employing genetic markers. Strains in which the cells are spherical instead of oblong, in which abnormal quantities of intracellular volutin are accumulated, or in which the flagella are paralyzed, have all been used in such experiments (Lewin, 1950). Observations on reciprocal combinations between suspensions of gametes labelled in these various ways have shown that it is the plus partner which, in the posterior position, is responsible for the propulsion of the pair. The minus partner, shortly after the formation of the intercellular bridge, ceases its flagellar activity (except for rare erratic bursts in the early stages of pairing). This difference in behavior is not dependent on the physiological state of the cells, but is genetically inherent in the mating types.

Summary

1. Cells of *Chlamydomonas moewusii* normally swim by a synchronized backward beat of the paired flagella.

2. When appressed to solid substrata, the flagella are capable of an independent creeping movement. This faculty does not persist in detached flagella.

3. During mating, cell clumps are formed by the adhesion of flagella of cells of opposite mating-types.

4. By means of the flagella, copulating cells can adjust their relative positions until cytogamy can be initiated.

5. Paired cells do not fuse for some hours, during which swimming results from the activity of one partner only.

6. By suitable labelling, it can be shown that the flagella of the plus partner remain active, while those of the minus cell cease to beat after pairing.

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TEMPERATURE REQUIREMENTS FOR MATURATION OF GONADS OF NORTHERN OYSTERS

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Gonad development and spawning of oysters (*Crassostrea virginica*) of our North Atlantic coast are well-defined seasonal phenomena. For example, in Long Island Sound, where extensive oyster beds are located, spring gonad development commences during the first part of May and spawning begins during the end of June or the first week in July (Loosanoff and Engle, 1942). There is no doubt that the rate of progress of gametogenic activities and the beginning of spawning of oysters are to a large extent influenced by the temperature of the surrounding water.

In the opinion of many students the period required for maturation of the gametes of the European oyster, *O. edulis*, is a function of time and temperature (Voisin, 1933; Orton, 1937; Korringa, 1941, and others). Few studies of a similar nature have been made, however, on the American oyster, *C. virginica*. Perhaps the only works dicussing effects of temperature on gonad development were those of Nelson (1928a), who thought that spawning of *C. virginica* should be expected approximately 160 degree-hours after the temperature of the water reaches and remains at the 20.0° C. level, and of Loosanoff (1942), who described seasonal gonadal changes of oysters of Long Island Sound and referred to the temperatures of the water prevailing during the different stages of gonad development and at spawning. In general, however, many aspects of the effect of temperature on the gonad development of *C. virginica* remained unknown.

The experiments described in this article were designed to ascertain the number of days needed for oysters of both sexes kept at different but constant temperatures to develop first mature cells; to reach the physiological state at which they can be induced to spawn by thermal or chemical means; and to reach the condition at which they will be ready to spawn normally, without any artificial stimulation. We hope that our article will contribute toward the understanding of the dependence of individuals and of populations upon ecological factors, such as temperature.

We wish to express our appreciation to our colleagues, Charles A. Nomejko, for assisting us in different phases of these studies, to David W. Calhoun for the statistical treatment of the data, and to Professor Thurlow C. Nelson of Rutgers University for reviewing the manuscript.

Method

The oysters used in these experiments ranged in age from three to five years. They were collected in winter when the temperature of the water over the beds was near 0.0° C. In the laboratory the oysters were separated into five groups and

placed in trays with running, cold sea water having a temperature of about 8.0° C., just high enough to allow the oysters to come out of hibernation. Twenty-four hours later the temperature of the water running in the trays was raised within 4 to 8 hours to 10.0, 15.0, 20.0, 25.0 or 30.0° C. and steadily maintained, within ± 1.0°C., at those levels until the end of the experiment.

In the first series of experiments small samples of oysters consisting of 5 to 15 specimens were taken at 5-day intervals for gross examination and histological studies (Loosanoff and Davis, 1949). In later experiments, however, each sample consisted of 50 oysters and when needed the oysters were examined daily. The quantitative data of this article are based on the latter samples.

The method of conditioning oysters to develop spawn in winter was, in principle, the same as that described several years ago (Loosanoff, 1945). It was modified only in the respect that instead of keeping the oysters in aquaria, the water of which was changed at certain intervals, they were kept in shallow trays through which water of the desired temperature was running continuously. The oysters were fed a rich culture of plankton which was automatically and continuously added to the running water.

Examination of the oysters was made according to the following procedure: Before the oysters were ripe enough to be induced to spawn, all 50 individuals constituting each sample were opened and the content of their gonads examined under a microscope to determine the number of males with active sperm, number of females with fertilizable eggs, and the number of immature oysters (Tables I–IV). All males containing active sperm were considered physiologically mature although not necessarily ready to spawn. Possession of fertilizable eggs was determined by adding mature sperm to the egg suspension made from each female containing large ovocytes. Formation of the polar body was taken as the criterion that the eggs were fertilizable.

After the oysters were kept long enough at the conditioning temperatures to be approaching the state in which they could be induced to spawn the procedure of the examination was changed as follows: Each of the 50 oysters constituting a sample was placed in a separate spawning dish, which was filled with water of the same temperature as that at which that group of oysters was conditioned. Then all the oysters were subjected to chemical stimulation consisting of the addition of sperm and egg suspensions to the water in the spawning dishes.

Ninety minutes after the beginning of the chemical stimulation all the oysters that failed to respond were further stimulated by an increase of the water temperature. After another period of 90 minutes the oysters that failed to spawn were opened and their gonads examined for the presence of active sperm or fertilizable eggs.

Observations

Control samples

Gonads examined at the beginning of the experiment contained small undeveloped follicles typical for hibernating oysters (Loosanoff, 1942). The male sex cells were in the early stages of spermatogenesis, predominantly spermatogonia or early spermatocytes. In the females the follicles contained mostly indifferent cells, some ovogonia and a few young ovocytes 15–18 μ in diameter. In both sexes most

of the space between the body wall and the digestive diverticula was occupied by the vesicular connective tissue which surrounded the small islands formed by the follicles (Fig. 1). The oysters were in good condition and contained large quantities of glycogen.

10.0° C. group

Even the most advanced oysters of this group, examined 35 days after the beginning of the experiment, showed only a slight development of their gonads. In the females the largest ovocytes were only 18–20 μ in diameter and contained virtually no yolk (Fig. 2). In the males, there were secondary spermatocytes and a few spermatids, but no spermatozoa. Even in these individuals, which constituted about 5 to 6 per cent of the samples, such development took place only towards the end of the experiment. In the other oysters of this temperature group the gonads even then resembled the winter condition.

It is interesting that in the 10.0° C. group the anastomosis of the follicles and gametogenesis were so slow even though these oysters, judging by the quantities of feces produced, were feeding more actively than those kept at the temperature of 25.0 or 30.0° C. Moreover, at the end of the experiment the meats of the low-temperature oysters still contained approximately as much glycogen as they had at the beginning. Considering these conditions, which indicated that the oysters were kept in a relatively favorable environment, it seems apparent that the depressed gonad development of those oysters was principally due to the low temperature.

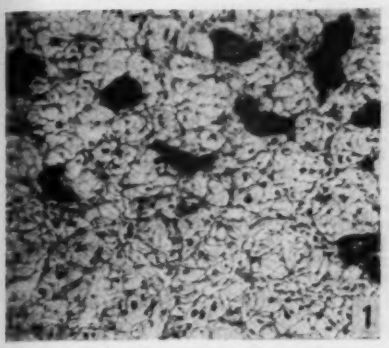
TABLE I

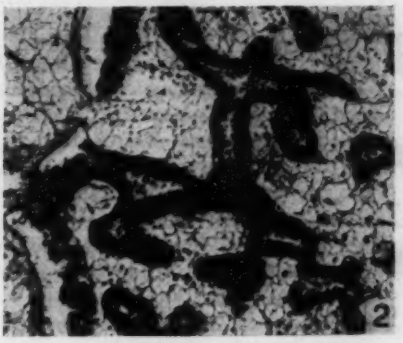
Number and per cent of oysters, kept for different periods at 15.0° C., that were induced to spawn, or showed presence of mature gametes. Number and per cent of immature oysters and those with sex undetermined are also given. Each sample composed of 50 oysters.

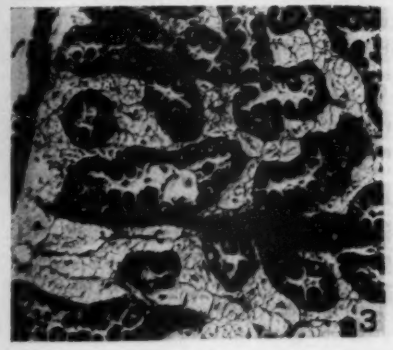
Days of conditioning	Induced to spawn				Unspawned				Per cent		
	By chemical stimulation		By chemical and thermal stimulation		Mature gametes		Immature gametes		Spawned	Unspawned but with mature gametes	Immature
	Male	Female	Male	Female	Active sperm	Fertilizable eggs	Sex recognizable but immature	Sex undetermined			
10	—	—	—	—	5	0	11	34	—	10	90
15	—	—	—	—	6	0	14	30	—	12	88
20	—	—	—	—	6	3	16	25	—	18	82
25	—	—	—	—	16	6	10	18	—	44	56
30	0	0	0	0	26	9	11	4	0	70	30
35	0	0	2	1	17	17	7	6	6	68	26
40	0	0	3	0	19	19	7	2	6	76	18
45	0	0	4	2	16	20	7	1	12	72	16
55	1	0	14	5	3	27	0	0	40	60	0

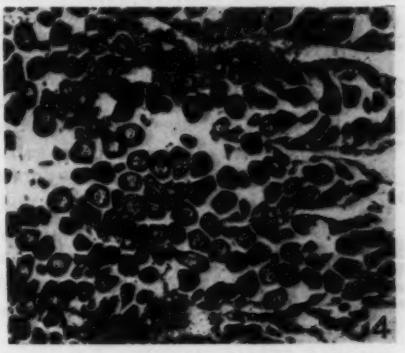
15.0° C. group

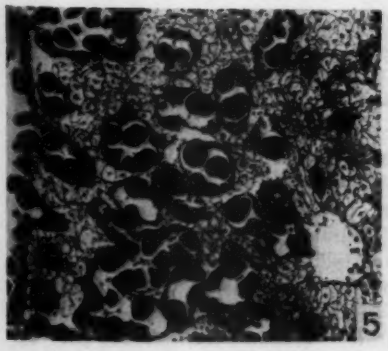
During the first five days of exposure the oysters showed little gametogenic activity and their gonads still remained in winter condition. The advanced males











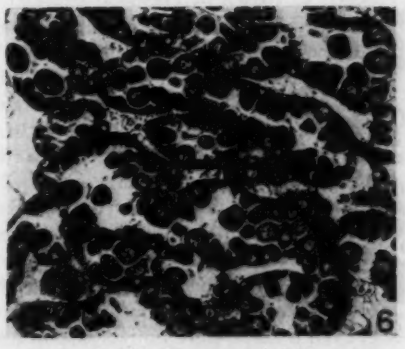


FIGURE 1. Winter gonad of oyster showing undeveloped follicles surrounded by connective tissue. × 112.

FIGURE 2. Gonad of most advanced female oyster of the group kept 35 days at 10.0° C. × 112.

FIGURE 3. Gonad of female oyster showing proliferating follicles containing growing ovocytes; 15 days at 15.0° C. × 112.

FIGURE 4. Gonad of female oysters; 40 days at 15.0° C. × 112.

FIGURE 5. Partly discharged gonad of female oyster; 55 days at 15.0° C. × 112.

FIGURE 6. Gonad of female oyster approaching ripeness; 13 days at 20.0° C. × 112.

showed a slight proliferation of follicles but gametogenesis proceeded only as far as the formation of spermatids. After 10 days the female oysters still showed little progress, while in the males a slight anastomosis of the follicles was noticed and in five of them a few spermatozoa were found (Table I). Towards the 15th day the largest ovocytes were already about 35 μ in diameter, but the vesicular connective tissue still occupied a large part of the interfollicular spaces (Fig. 3).

By the 20th day 9 oysters, representing 18 per cent of the sample, contained active sperm or fertilizable eggs, while 16 other oysters possessed recognizable sex cells (Table I). Thus, on this date the sex of 50 per cent of the oysters composing the sample was already recognizable.

On the 25th and 30th days attempts were made to induce the oysters to spawn. They were first stimulated by the addition to the water of a suspension of ripe spermatozoa or eggs, and later by a rapid increase of the temperature to about 32.0° C. Some oysters, probably females, moved their shells in typical rhythmic spawning motions, but no eggs were discharged.

Spawning was successfully induced for the first time on the 35th day (Table I). However, of 50 oysters composing the sample only three, two males and one female, spawned and then only after the combined effects of chemical and thermal stimulations. The eggs obtained in this spawning developed into normal larvae.

By this time a difference in the gonad development of the individuals composing the samples became especially pronounced. The thickness of the gonadal layer varied from almost 0.0 to 3.0 mm. and while the most advanced oysters could be induced to spawn, the least advanced possessed gonads so undeveloped that their sex could not be easily determined (Table I).

Between the 35th and 45th days the oysters continued to develop, their enlarged gonadal follicles coming in contact with each other, while the vesicular connective tissue, which was so prominent earlier in the development, had largely disappeared (Figure 4). Yet, even on the 45th day none of the oysters could be induced to spawn by chemical stimulation alone.

After 55 days all the oysters had either fertilizable eggs or active sperm, and 20 of them, constituting 40 per cent of the sample, were induced to spawn (Table I). Of these, however, only one responded to chemical stimulation alone, while all others required the rapid increase in temperature. It is possible that this happened because several oysters in the sample had already discharged some spawn, and were, therefore, comparatively indifferent to the stimulating effect of sex products. That some of the oysters may have spawned was shown by histological examination of the gonad tissue taken from the oysters of an auxiliary experimental group, some of which showed partially discharged gonads, characterized by contracting follicles and the invasion of connective cells into the interfollicular spaces (Fig. 5).

At the end of the 90-day period an egg and sperm suspension was added to the trays to determine if the oysters would respond to this stimulation and begin spawning, even if there were no increase in temperature. Within a few minutes after the addition of the suspension almost all the oysters in the tray commenced spawning and continued to do so for about two hours. During the spawning the temperature steadily remained at 15.8° C. The eggs collected from this spawning developed into normal larvae.

20.0° C. group

The stimulating effect of this temperature was clearly seen early in the experiment. At the end of the first five days 28 per cent of the oysters already contained either active sperm or fertilizable eggs (Table II). After eight days the oysters were first induced to spawn by a combination of chemical and thermal stimuli, and two days later some oysters responded to chemical stimulation alone (Table II). Although some ripe eggs were found in the females after only five to ten days of exposure, nevertheless, such eggs still represented a minority of the follicular cells.

TABLE II

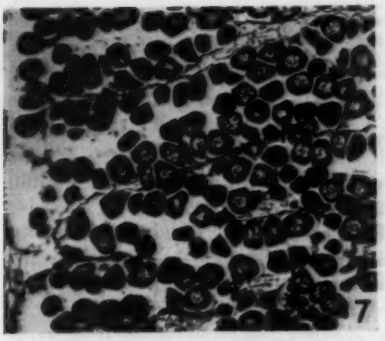
Number and per cent of oysters, kept for different periods at 20.0° C., that were induced to spawn, or showed presence of mature gametes. Number and per cent of immature oysters and those with sex undetermined are also given. Each sample composed of 50 oysters.

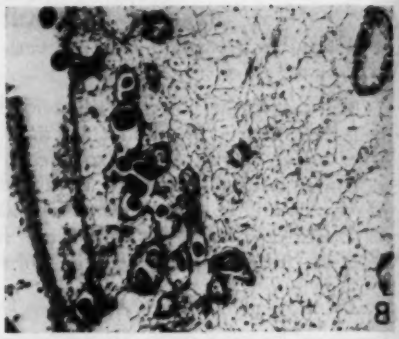
Days of conditioning	Induced to spawn				Unspawned				Per cent		
	By chemical stimulation		By chemical and thermal stimulation		Mature gametes		Immature gametes		Spawned	Unspawned but with mature gametes	Immature
	Male	Female	Male	Female	Active sperm	Fertilizable eggs	Sex recognizable but immature	Sex undetermined			
5	0	0	0	0	9	5	7	29	0	28	72
8	0	0	2	0	14	4	18	12	4	36	60
10	2	0	11	0	9	7	14	7	26	32	42
13	1	0	7	8	8	16	9	1	32	48	20
15	3	3	9	6	4	14	10	1	42	36	22
18	6	6	3	11	8	12	2	2	52	40	8
20	1	1	16	12	1	18	1	0	60	38	2
25	0	0	19	6	4	21	0	0	50	50	0

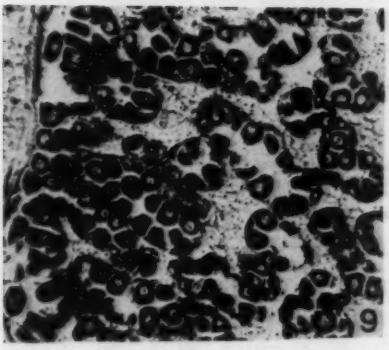
Again, as in the preceding temperature classes, the individual differences among the oysters of the same group were striking. While 26 per cent of the oysters kept at 20.0° C. for ten days could be induced to spawn, 7 others still possessed gonads resembling the winter condition (Table II). This condition of slow gonad development persisted even though the oysters were feeding vigorously and showed rapid growth of shell. Similar individual differences in gonad development were also found in oysters growing under natural conditions in Long Island Sound (Loosanoff, 1942).

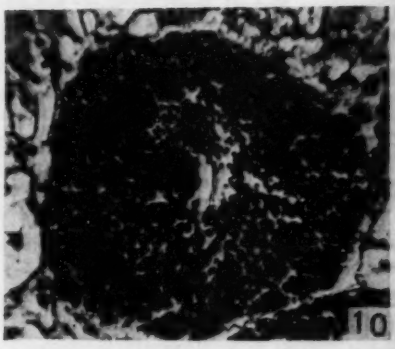
The spawning behavior of the females within the samples also showed that they were in physiologically-different states of ripeness. Some of them did not respond at all to stimulation. These were usually immature individuals, the sex of which could not be determined. Others moved their shells as in spawning but released no eggs. Still others discharged a few apparently immature eggs measuring only 45 μ, *i.e.*, about 5 μ smaller than normal eggs. Finally, there were females that discharged normal eggs which developed into healthy larvae. The ratio between these groups changed continuously, as can be judged from the data in Table II.

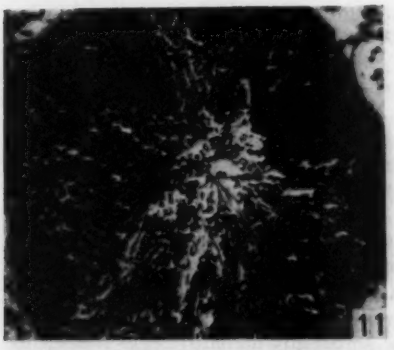
Between the 13th and 18th days many oysters were either approaching ripeness (Fig. 6) or were ripe (Fig. 7). At the latter date 52 per cent of the oysters











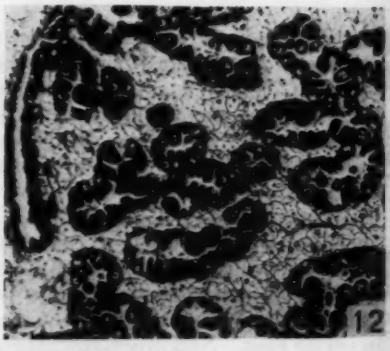


FIGURE 7. Gonad of ripe female oyster; 18 days at 20.0° C. × 112.

FIGURE 8. Gonad of female oyster in advanced stage of spawning; 25 days at 20.0° C. × 112.

FIGURE 9. Gonad of female oyster containing some ripe eggs but still not quite mature; 8 to 10 days at 25.0° C. × 112.

FIGURE 10. Gonadal follicle of male oyster showing few ripe spermatozoa in center of lumen; 3 days at 30.0° C. × 475.

FIGURE 11. Gonadal follicle of male oyster; 5 days at 30.0° C. × 475.

FIGURE 12. Gonad of female oyster containing some fertilizable eggs; 5 days at 30.0° C. × 112.

spawned, when stimulated, and the number of individuals with sex undetermined was only one or two per sample of 50. After 20 days 98 per cent either spawned or contained ripe gametes.

On the 20th day of exposure the number of oysters that could be induced to spawn showed a considerable decrease, as compared with the record of the 18th day (Table II). Between these two dates some of the oysters had apparently spawned in the natural, unprovoked way, and the presence of their sex products in the surrounding water may have made them unresponsive to this type of stimulation when it was applied experimentally a day or so later. This contention is supported by the observation that on the 25th day of the experiment none of the oysters could be induced to spawn by chemical stimulation alone. Histological studies of their gonads also showed that some were already in the typical post-spawning condition characterized by contracted follicles still retaining some ripe eggs, a large number of phagocytes, and a rapid invasion of connective tissue throughout the space between the body wall and the digestive diverticula (Fig. 8). Such oysters, obviously, could not be easily stimulated and, therefore, were not included in the number of oysters induced to spawn on that date. Instead, they were classified together with those that were unspawned but contained active sperm or fertilizable eggs. This explains why on the 25th day of exposure the number of oysters that could be induced to spawn showed a decrease, while the number of individuals which were unspawned but contained ripe gametes showed an increase (Table II).

25.0° C. group

In this group rapid gametogenesis and proliferation of gonadal follicles began within the first few days. After five days 17 oysters, constituting 34 per cent of the sample, already contained mature gametes (Table III). Of these 13 were males containing active sperm and the rest were females with fertilizable eggs. In both sexes, however, there were only a few ripe cells, indicating that the oysters were just entering the state of ripeness.

TABLE III

Number and per cent of oysters, kept for different periods at 25.0° C., that were induced to spawn, or showed presence of mature gametes. Number and per cent of immature oysters and those with sex undetermined are also given. Each sample composed of 50 oysters.

Days of conditioning	Induced to spawn				Unspawned				Per cent		
	By chemical stimulation		By chemical and thermal stimulation		Mature gametes		Immature gametes		Spawned	Unspawned but with mature gametes	Immature
	Male	Female	Male	Female	Active sperm	Fertilizable eggs	Sex recognizable but immature	Sex undetermined			
5	—	—	—	—	13	4	10	23	—	34	66
6	0	0	0	0	19	8	11	12	—	54	46
7	0	0	8	4	7	13	10	8	24	40	36
8	5	1	3	1	13	17	10	0	20	60	20
9	1	3	7	5	8	13	9	4	32	42	26
10	9	13	3	5	5	10	3	2	60	30	10

By the end of the seventh day some of the oysters were induced to spawn by the combined effects of chemical and thermal stimuli, and the next day five males and one female responded to chemical stimulation alone.

After 9 and 10 days of conditioning the samples gave 32 and 60 per cent of spawners respectively, and at the latter date the number of immature oysters was only 10 per cent (Table III). However, some of those that were capable of spawning still possessed gonads containing many immature cells. This condition is well illustrated in Figure 9, where together with mature ovocytes there are many unripe cells. Furthermore, the large quantity of connective tissue in the interfollicular spaces indicated that further growth of the follicles was still to occur.

Mass spawning of the oysters of this temperature group took place during the eleventh day. In some earlier experiments, however, mass spawning of one group kept at 25.0° C. occurred on the ninth day (Loosanoff and Davis, 1949). In both cases the spawning was unprovoked since the temperature remained steadily at 25.0° C. and no sex products capable of stimulating the experimental oysters could be present in the water flowing into the trays, because the experiments were conducted in February when the oysters in the Harbor, from which our water supply is obtained, were hibernating and, obviously, could not release any spawn.

30.0° C. group

This group, as a whole, responded extremely rapidly to the effect of high temperature and after three days of exposure 18 per cent of the oysters already possessed physiologically-ripe cells (Table IV). Although the most advanced females showed expanding follicles already containing a few fertilizable eggs, their gonads, nevertheless, still had a large quantity of connective tissue separating the follicles. In the males rapid spermatogenesis was in progress, already resulting in a few ripe spermatozoa in the centers of the lumens of the follicles (Fig. 10).

TABLE IV

Number and per cent of oysters, kept for different periods at 30.0° C., that were induced to spawn, or showed presence of mature gametes. Number and per cent of immature oysters and those with sex undetermined are also given. Each sample composed of 50 oysters.

Days of conditioning	Induced to spawn				Unspawned				Per cent		
	By chemical stimulation		By chemical and thermal stimulation		Mature gametes		Immature gametes		Spawned	Unspawned but with mature gametes	Immature
	Male	Female	Male	Female	Active sperm	Fertilizable eggs	Sex recognizable but immature	Sex undetermined			
3	—	—			7	2	3	38	—	18	82
4	—	—			26	3	6	15	—	58	42
5	16	3	Not		7	3	8	13	38	20	42
6	15	12	Employed		10	1	8	4	54	22	24
7	22	13			1	2	6	6	70	6	24

After five days the oysters were successfully induced to spawn. Because they were conditioned at the temperature of 30.0° C. it was impractical to use a much higher temperature to induce spawning and, therefore, chemical stimulation

alone was used. Nineteen oysters of the total number of 50 spawned but the quantities of spawn released were still small. Histological examination of the gonads showed that although the males conditioned at 30.0° C. for five days (Fig. 11) had considerably more mature sperm than after three days (Fig. 10), the unripe cells, nevertheless, still predominated. The same could be said for the females in which ripe ovocytes were not too common (Fig. 12).

After six days 54 per cent of the oysters were induced to spawn. Yet, 24 per cent were still immature and in four per cent the sex could not be determined (Table IV). After seven days 70 per cent could be spawned, and in one of the experiments an unprovoked mass spawning took place at the end of the seventh day. Both sexes participated in the spawning, the males discharging large quantities of sperm, but the number of eggs released still remained comparatively small.

By the tenth day the gonads of the oysters were already in advanced post-spawning stages, characterized by contracted follicles similar to those shown in Figures 5 and 8.

Discussion

As has already been mentioned, in studying the maturation of gonads of oysters kept at different but constant temperatures we were primarily interested in the number of days needed for formation of the first physiologically-mature gametes in each sex. We were also interested in finding how soon oysters can be induced to spawn by artificial means, *i.e.*, by the addition of sex products or by a rapid increase in temperature and, finally, how soon they would begin to spawn normally, without the help of any artificial stimulation.

Our experiments showed that the temperature of 10.0° C. is not high enough to induce gametogenic activities in most of the oysters, and in those few in which such activities were initiated, the development proceeded at an extremely slow rate. Apparently this temperature was too low to permit the conversion of glycogen and other materials stored in the oyster bodies into sex products. While the exact mechanism of this complex process is still not understood, it is thought that it involves the action of a certain enzyme or chain of enzymes, which is retarded, or completely inhibited, at low temperatures.

There are ample indications that some processes in animals, including oysters, are slowed down by low temperature more than others, thus showing that the effect of such low temperature is, obviously, differential. For example, while the growth of the shell of the oysters of our waters may proceed even at a temperature of one or two degrees below 10.0° C., the gonad development at such a temperature is either entirely arrested or proceeds at such a slow rate that a successful completion of that process cannot be expected. Perhaps this is a good example of the so-called developmental or biological zero of Bĕlehrádek (1935) who considers such a zero as the highest temperature threshold at which a certain protoplasmic activity is still arrested by cold.

Our observations, that at a temperature of 10.0° C. even the most advanced oysters did not form spermatozoa after 35 days of exposure, differ from those of Nelson (1928b) who, in his studies of oysters in Barnegat Bay, found that "active spermatozoa may be found in a few oysters at temperatures as low as 9°–10° C., Table I." Nelson's table shows, however, that the temperature data which he

offers were the averages for the week preceding the date of collection of the gonad samples. It is possible, therefore, that the maximum temperature during, or prior to, this period exceeded 10.0° C., causing spermatogenic activities which resulted in the formation of a few spermatozoa. Nevertheless, the results of our controlled experiments are in full agreement with the field observations of Nelson (1928a) who emphasized the wide individual differences often found in the condition of the gonads of oysters collected at the same time and from the same bed.

The experiments also demonstrated that under certain conditions ripening of gonads and spawning of oysters of both sexes may be accomplished at a temperature of only about 15.0° C. These experiments prove rather conclusively that the old conception that 20.0° C. is the minimum temperature at which spawning of *C. virginica* is possible does not hold for the oysters of Long Island Sound.

Laboratory observations on ripening of gonads at such a relatively low temperature corroborate the studies in Long Island Sound, which showed that spawning of oysters may take place at a temperature as low as 16.4° C. (Loosanoff, 1939). Recently we have found that in nature oysters may possess ripe gonads in early summer when the temperature of the surrounding water is only 14.6 to 15.4° C. On July 1, 1948 samples of oysters were taken from two of our collecting stations located in Long Island Sound at a depth of 30 feet. At the time of collection the temperature of the water at the first station was 14.3° and at the second, 15.4° C. The highest temperature recorded that summer at the first station, prior to collection of the sample, was 14.6° C., while at the second station it was 15.4° C. These oysters were placed in the laboratory in separate trays of slowly running water at about 22.0° C. A few hours later the oysters began to spawn copiously, both sexes participating in the act. The fertilized eggs were cultured and became normal larvae.

A similar experiment was repeated in early June, 1949. The oysters were collected from a depth of 35 feet, where the highest temperature recorded for the season was only 14.5° C. Again the oysters spawned a few hours after they were placed in warm water. Thus, the oysters were near the spawning condition at the time of collection even though the highest temperature the water had reached that season was only about 15.0° C.

Since spawning is possible at temperatures lower than 20.0° C., Nelson's (1921) conclusion that if the temperature of the water fails to reach 70.0° F., (21.2° C.) and remain at that level for some time, the oysters will not spawn at all, should be regarded as not applicable to the oysters of Long Island Sound. For the same reason Nelson's (1928a) suggestion that spawning of *C. virginica* may be expected to begin approximately 160 degree-hours after the temperature of the water has reached 20.0° C. is not true for Long Island Sound oysters. We think that the discrepancies between our observations and Nelson's may be explained on the basis that the two studies were made in geographical areas where ecological conditions are different, and where the local populations of oysters may possess significantly different physiological traits. That such physiologically-different groups of oysters may exist was recently shown by Stauber (1950) who by reviewing published and some unpublished records concluded that there appear to be three physiological races of oysters, *C. virginica,* which require different minimum temperatures at which they are capable of spawning. Stauber showed that the race with the lowest critical temperature is not found in Canada, at the northern limit of the

geographical range of the species, but in Long Island Sound, some 600 miles airline farther south. Loosanoff and Nomejko (1951), by conducting experiments in Milford Harbor, Connecticut, with oysters brought from different geographical areas along the Atlantic coast, found that the breeding temperature requirements of the northern oysters were somewhat lower than those of the southern groups, thus corroborating Stauber's conclusions and suggesting once more that some physiological requirements of oysters of different geographical districts may be decidedly different. We would also like to mention that our colleague, Professor Thurlow C. Nelson, called our attention to the most interesting fact that many years ago Lamarck from shell characteristics alone designated the Long Island Sound oyster as *Ostrea borealis,* which he believed to be a species distinctly different from *O. virginica* (now *Crassostrea virginica*).

TABLE V

Per cent of oysters of both sexes with mature gametes and estimated number of females in samples of 50 oysters kept at temperature of 15.0, 20.0, 25.0 or 30.0° C. for different numbers of days. Calculated days' exposure compensated for inequalities in sex ratio are also shown.

Days exposed	Temperatures in °C.											
	15.0			20.0			25.0			30.0		
	Compensated days exposed	% Oysters with mature gametes	Estimated number of females	Compensated days exposed	% Oysters with mature gametes	Estimated number of females	Compensated days exposed	% Oysters with mature gametes	Estimated number of females	Compensated days exposed	% Oysters with mature gametes	Estimated number of females
3										3.0	18	27
4										5.2	58	18
5				4.9	28	28	4.9	34	28	5.7	58	23
6							6.1	52	27	6.7	76	24
7							6.1	64	33	7.6	76	25
8				7.8	40	29	7.8	80	29			
9							7.9	74	33			
10	9.8	10	29	10.6	58	26	8.8	90	33			
13				11.2	80	34						
15	14.5	12	30	12.9	78	34						
18				16.1	92	33						
20	17.4	18	34	18.4	98	32						
25	25.9	44	28	26.6	100	27						
30	35.9	70	23									
35	35.6	74	29									
40	43.2	82	27									
45	44.9	84	30									
55	52.3	100	32									

As is well known, the rate of metabolic processes of an animal increases almost to the extreme upper limit of the temperature at which this animal carries on its activities. This is especially well noticed in poikilothermous animals, the body temperature of which is usually at or near that of their environment. To express more or less accurately the influence of temperature on biological phenomena and to demonstrate the relation existing between temperature and the speed of physiological

processes, a number of formulae have been proposed by many students including the most often quoted Krogh (1914), Arrhenius (1915) and Bělehrádek (1935). It is not the purpose of our article to evaluate the relative merits of the different formulae, except to mention that although there is not a universally acceped mathematical expression for the effect of temperature upon the rate of biological processes, it is widely recognized, nevertheless, that some of the formulae, including those mentioned above, offer biologists a tool for an approximate solution of the problems and, at times, for prediction of the results of the studies concerning relationships between temperature and physiological processes, such as gonad development or spawning.

Choice of indices of the gonadal development of a group of oysters is somewhat arbitrary. The two most satisfactory for examination were the number of individuals with active sperm and the number with fertilizable eggs. Accordingly, an analysis was made of the sums of these two indices, as a percentage in each sample of 50 oysters (Table V). This percentage is hereafter called the degree of maturity of the sample. It was then transformed to the probit scale, and compared with the exposure time on a logarithmic scale. The four groups of samples at the different temperatures gave four series of points. By the method of maximum likelihood four parallel straight lines with the slope 4.343 were fitted, one to each series. Deviations did not appear to come from systematic curvature or non-parallelness of the fitted lines.

This analysis indicated that (1) since straight lines follow use of the log-time scale, individual differences may be explained by variability in the rates of development of different individuals. (2) Parallelism of the four lines is evidence that a given temperature increase affects all individuals in the same way, accelerating their rates of development by the same factor. (3) The slope of the fitted lines measures the variation of the oyster population with respect to differences in time required to reach maturity. For example, at a given temperature, it will take the slowest developing 20 per cent of the population over 2.5 times as long to reach maturity as it does the fastest 20 per cent (Fig. 13).

Our experiments have shown that males mature earlier than females (Tables I–V). Therefore, we would expect that samples largely female would be slow and those predominantly male would be farther advanced than the average. Since the sex of oysters with poorly developed gonads could not be recognized, the actual number of each sex in some samples was unknown. Hence, the sexes could not be analyzed separately, and random fluctuations in sex ratio have presumably disturbed the temperature averages, making comparison uncertain. A refinement of the analysis was obtained by allowing for the estimated proportion of females in each sample. An appropriate allowance was then subtracted from the actual time for predominantly female samples and added to the time of the samples with more than the average number of males.

Estimated sex ratios in each sample were based on the preliminary fitted lines, by dividing the number of sexually indeterminate individuals roughly in proportion to the expected unripe fractions for each sex. The resulting estimate, expressed as number of females, is shown in Table V. Based on these estimates an allowance was calculated, using partial regression, leading to compensated exposure times: $x = 1.03035x_1 - .01212x_2 + .31226$, where x_1 is the original log-time, x_2 is the

estimated number of females in the sample and x is the log of the compensated time. The latter is the time that would presumably be required for a sample of 50 oysters containing 28.37 females, which is the weighted average for the experiment, to reach the same degree of maturity as the given sample. This is presented in Figure 13, showing degree of maturity plotted against compensated exposure time, with

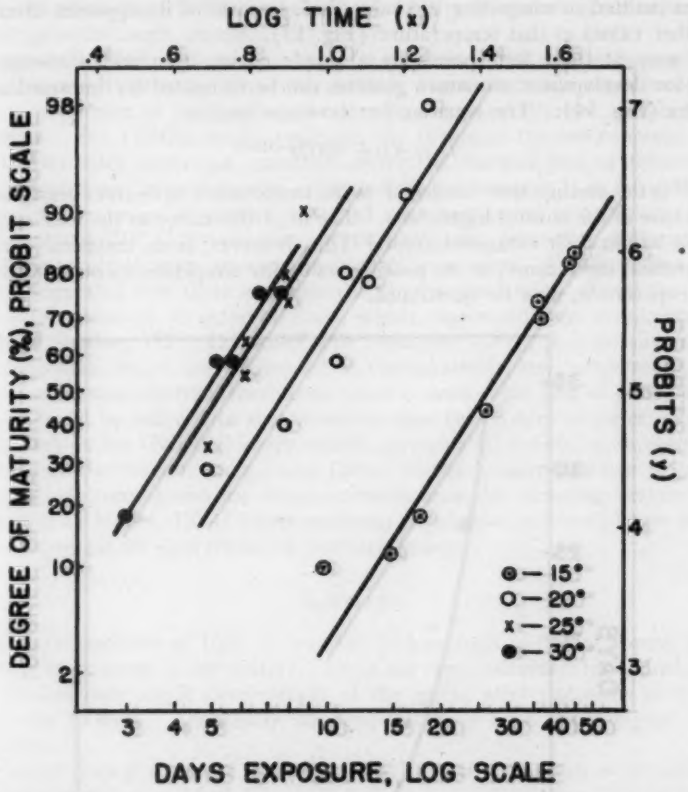


FIGURE 13. Degree of maturity (%) in samples of 50 oysters plotted against period of exposure to four experimental temperatures. Each period of exposure is compensated for the estimated sex ratio of the sample.

a straight line of slope 4.343 fitted for each of the four temperatures. This fit is satisfactory after compensation for sex ratio: chi-square equals 30.8, with 22 degrees of freedom, which is just at the 10 per cent level of significance.

From the equation for each line, $y - \bar{y} = 4.343\ (x - \bar{x})$, where y is the probit of degree of maturity and $\bar{x}$ and $\bar{y}$ are the average values of x and y for a given temperature, the average time required for 50 per cent of oysters to develop mature

gametes can be calculated for each temperature. This corresponds to the value of x when $y = 5$. For the four temperatures this average time was:

15.0° C. – 26.5 days	25.0° C. – 5.4 days
20.0° C. – 7.9 days	30.0° C. – 4.9 days

Their error is estimated as about 10 per cent. The lowest observation at 15.0° C. has been omitted in computing the value 26.5, because of its apparent discrepancy from other values at that temperature (Fig. 13).

We may fit these four points by a simple curve, from which average times needed for development of mature gametes can be estimated for intermediate temperatures (Fig. 14). The equation for the curve used is:

$$D = 4.8 + 4205e^{-.3554T}$$

where D is the average time needed, T is the temperature in degrees centigrade, and e is the base of the natural logarithms. The fit of this curve to the four established points is within their estimated error. This, however, is an empirical curve, and its theoretical significance, or its possible value for temperatures outside the range of the experiment, may be questioned.

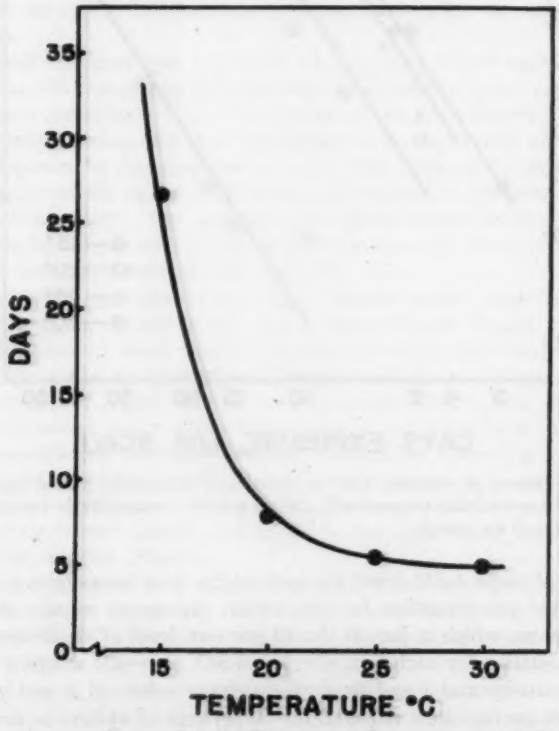


FIGURE 14. Average time needed for 50 per cent of an oyster population to develop mature gametes at different temperatures.

We have noticed that the quantity of glycogen contained by the oysters at the beginning of gonad development may control the quantity of spawn that will be produced. If the oysters are "poor," *i.e.*, containing little glycogen, they usually do not develop good gonads. For example, our attempts to condition for spawning in winter several groups of oysters shipped to us from the Florida waters of the Gulf of Mexico failed because those oysters were thin and watery, containing virtually no glycogen. Similar experiments with poor oysters from other bodies of water gave the same results.

In concluding this discussion it may be mentioned that the knowledge of the thermal history of the organisms prior to their exposure to the experimental conditions is important in interpreting the results of the experiment and in forming conclusions. Fry (1947), in his paper on the effects of the environment on animal activities, cites numerous examples where the thermal past of fishes reflects profoundly on their behavior under experimental conditions. It is possible, therefore, that if the groups of oysters used had been acclimated to a higher temperature than almost 0.0° C., which was our starting point, somewhat different results would have emerged from an experiment similar to ours. Furthermore, since it has been suggested that there are different physiological races among the population of *C. virginica* of our Atlantic coast, which require different temperatures for spawning (Stauber, 1950; Loosanoff and Nomejko, 1951), it is possible that our northern oysters, which are acclimated to comparatively low temperature, would, under the same experimental conditions, show a more rapid rate of gonad development and could be induced to spawn earlier than the oysters of other geographic regions, such as the Gulf of Mexico which, ecologically, maintains an entirely different temperature regime from Long Island Sound. Such relations to temperature conditions were found for several aquatic animals, including certain species of amphibians (Moore, 1939) where northern populations survived lower temperatures and bred earlier than the more southern groups.

Summary

1. The temperature of 10.0° C. was not high enough to induce normal gametogenic activity in most of the oysters. Even the most advanced individuals of this group showed only slight development of the gonad after exposure to this temperature for 35 days. Apparently this temperature is near the biological zero for this activity.

2. Ripening of gonads and spawning of oysters of both sexes were achieved at a temperature as low as 15.8° C.

3. Wide individual differences in the extent of gonad development among the individuals constituting the same groups were commonly found.

4. In all temperature groups, ranging from 15.0 to 30.0° C., the physiologically ripe gametes were generally formed earlier in the males than in the females.

5. At 15.0° C. the most advanced males contained a few ripe spermatozoa on the 10th day. Fertilizable eggs were found on the 20th day and spawning was induced by the 35th day. At 20.0° C. oysters with ripe spermatozoa and fertilizable eggs were found on the fifth day. Spawning was induced on the 10th and 13th days in males and females respectively. At 25.0° C. ripe spermatozoa and fertilizable eggs were found by the fifth day. Spawning was induced on the seventh day. At 30.0° C. ripe spermatozoa and a few fertilizable eggs were found three

days after the hibernating oysters were taken from their winter environment and placed at this temperature. Spawning was induced on the fifth day.

6. The average time at each of the experimental temperatures required for 50 per cent of the oysters to develop mature gametes was calculated to be:

15.0° C. – 26.5 days	25.0° C. – 5.4 days
20.0° C. – 7.9 days	30.0° C. – 4.9 days

To estimate the average time needed for development of mature gametes at temperatures intermediate to those given above, a simple curve is offered based on the equation:

$$D = 4.8 + 4205e^{-.3554T}$$

where D is the average time needed, T is the temperature, and e is the base of the natural logarithms.

7. The quantity of glycogen in the oysters at the beginning of gonad development may control the quantity of spawn produced.

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THE EFFECTS OF ETHYL ALCOHOL ON GROWTH AND RESPIRATION IN PELOMYXA CAROLINENSIS

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Among the first of comparatively few studies made on the effect of alcohol on unicellular organisms were those of Calkins and Lieb (1902) and Woodruff (1908). These workers used Paramecium and in general concluded that ethyl alcohol, in "moderate" concentration, acts as a stimulus in sustaining the vitality of these cells.

On the contrary, Matheny (1910) and Estabrook (1910) found no evidence that alcohol acts as a periodic or continued stimulus. In minute doses, 2% or less, it apparently has no effect on Paramecium while in doses of 3% or greater, it kills them. Daniel (1909) working with another genus, Stentor, found that ethyl alcohol causes an increase in growth rate. He also states that comparatively weak solutions of alcohol (1% or less) are ineffective, while a 4% solution causes early death.

More recently Bills (1924) observed that alcohol seems to restore starved paramecia to their former vitality. Loefer and Hall (1936) studied the effects of alcohol on several species of ciliates and flagellates. Of those studied, growth was accelerated in only two species, *Euglena gracilis* and *Euglena deses* (in 0.025 to 0.1% alcohol). Goldschmied-Hermann (1935) found that Paramecium dies in 3% ethyl alcohol. In concentrations of 1 to 2%, movements become sluggish and there is an increase in number of vacuoles and in the rhythmicity of their contractions. Three or four vacuoles were occasionally formed instead of the usual two.

Even fewer studies have been made on amoeboid cells and their reactions to alcohol. Brinley (1928) worked with Amoeba and found that 5% ethyl alcohol causes them to retract their pseudopods and take on a spherical form. The protoplasm becomes very fluid. Frederikse (1932) confirmed these results.

Daugherty (1937) investigated the effect of methyl (1.99 *M*), ethyl (1.028 *M*), propyl (0.467 *M*), butyl (0.164 *M*), and amyl (0.066 *M*) alcohols on *Amoeba proteus* and *Amoeba dubia*. In weak concentrations a liquefaction of the plasmagel occurs at first but with prolonged exposure to these concentrations the liquefied portion becomes gelated. In medium or strong concentrations of these alcohols, gelation of the plasmasol was observed.

Because of rather limited knowledge on the effects of alcohol on living cells and because of the ease with which Pelomyxa lends itself to studies of this type, the following investigations were carried out.

Materials and Methods

The organism used in these investigations, *Pelomyxa carolinensis* Wilson (also referred to as *Chaos chaos, Chaos carolinensis* and *Amoeba carolinensis*), was

grown in a solution developed by Pace and Belda (1944). The medium was composed of the following: K_2HPO_4 – 80 mg.; KH_2PO_4 – 80 mg.; $CaCl_2$ – 100 mg.; $Mg_3(PO_4)_2 \cdot 4H_2O$ – 2 mg.; and redistilled water to 1000 ml.

Paramecium caudatum was used as food for the pelomyxae; the latter are from 25 to 50 times as large as the former. The paramecia were grown in comparatively narrow jars (500 cc. capacity) containing the above medium plus hay infusion. A week or ten days after addition of the paramecia to a fresh culture they may be collected in abundance from the surface. Stacking dishes (finger bowls) of 10 cm. diameter, to which 150 cc. of pelomyxa solution or test solution were added, were used as culture chambers.

In ascertaining growth, both numbers of organisms and their volumes were taken into consideration. The volumes were measured by means of a volumescope (Chalkley, 1929; Belda, 1942). In these experiments the over-all average volume varies very little, except in the highest concentration of alcohol.

The rate of oxygen consumption was ascertained by means of a Barcroft-Warburg respirometer.

All the various dilutions were made from freshly prepared culture media and 100% ethyl alcohol, which was the only alcohol tested. The solutions were changed daily in an attempt to maintain the alcohol concentrations as constant as possible.

Results

1. Effect of alcohol on growth in Pelomyxa

Ethyl alcohol was diluted with the culture solution, as previously described, to the following concentrations: 0.001 *M*, 0.005 *M*, 0.01 *M*, 0.05 *M*, 0.1 *M* and 0.5 *M*. In each experiment, three culture dishes were used for each concentration and 100 ml. of solution containing 25 pelomyxae were placed in each as well as in the control cultures (no alcohol). Each culture received one ml. of washed and concentrated paramecia every second day thereafter. The stacking dishes were sealed with Lubriseal to prevent possible loss of alcohol by diffusion from the culture.

The results of four experiments are presented in Table I. It is evident that 0.5 *M* alcohol is toxic to Pelomyxa. In every experiment there was a decrease in the number of organisms placed in this concentration and in Experiment 4 they died out altogether by the eighth day. In 0.005 *M* alcohol, growth is 29% greater than in the control cultures; it is also greater in 0.01 *M* and 0.05 *M* alcohol (12% greater in both) but higher concentrations than these have a retarding effect if they do not actually kill the organisms.

2. Effect of alcohol on structure, size and activity of Pelomyxa and upon digestion of food

Other characteristics, aside from growth, are also affected by alcohol. For example, at the end of 24 hours in solutions containing 0.5 *M* alcohol, only rarely is a pelomyxa found attached to the substratum. However, in the control and in the 0.001 *M* and 0.005 *M* alcohol solutions, they seemed to be firmly attached.

The organisms are also quite active in these solutions although in 0.01 *M*, 0.005 *M*, and 0.001 *M* alcohol they are much less active and even slight jarring of the culture dish will easily dislodge them if they are attached. In spite of these differ-

TABLE I

Growth of Pelomyxa in various concentrations of ethyl alcohol

Experiment No.	Molar concentration of alcohol	No. of pelomyxae on days (as designated) following inoculation					Total increase
		2	4	6	8	10	
1	0	34	39	54	127	208	183
	0.001	32	40	52	109	173	148
	0.005	29	41	62	145	276	251
	0.01	32	38	50	131	219	194
	0.05	30	44	65	170	252	227
	0.1	26	28	32	32	34	9
	0.5	23	22	22	18	10	−15
2	0	30	34	51	107	151	126
	0.001	33	46	67	116	192	167
	0.005	36	47	71	128	203	178
	0.01	34	48	63	136	224	199
	0.05	34	45	75	141	211	186
	0.1	29	37	49	72	108	83
	0.5	26	28	26	15	5	−20
3	0	28	34	84	177	262	237
	0.001	28	35	76	183	246	221
	0.005	29	36	99	265	342	317
	0.01	29	36	77	231	321	296
	0.05	26	32	99	257	328	303
	0.1	27	29	72	138	272	247
	0.5	26	24	22	19	18	−7
4	0	35	52	74	199	271	246
	0.001	27	49	74	177	253	228
	0.005	33	41	89	208	305	280
	0.01	29	44	74	162	224	199
	0.05	30	42	68	139	204	179
	0.1	25	25	32	47	91	66
	0.5	18	10	6	0	0	−25
Average for all experiments. (32 cultures for each concentration)	0	32	40	88	152	223	198
	0.001	30	42	87	148	218	193
	0.005	32	41	80	186	281	256
	0.01	31	41	88	185	247	222
	0.05	30	41	77	177	248	223
	0.1	27	30	48	72	126	101
	0.5	23	21	19	13	8	−17

Eight cultures were used for each concentration in each experiment; 25 pelomyxae were added to each culture; counts were made every two days; temperature, 25° ± 1° C.

ences between the experimental and control animals growth is greater in 0.01 *M* and 0.05 *M* alcohol solutions.

Pelomyxae exposed to high concentrations of alcohol, especially 0.5 *M*, are very inactive and appear to have a narrow solated area. The pseudopods are short and rounded. Very few food vacuoles are found, although numerous dead paramecia

are scattered over the bottom of the dish. In Figure 1, a typical normal pelomyxa is compared with one grown in 0.5 *M* alcohol solution.

One of the most striking structural changes is found in the hyaline layer, which in normal organisms is a narrow clear area (except at the tips of pseudopods, where it is quite pronounced) found immediately beneath the plasmalemma. In pelomyxae exposed to 0.5 *M* alcohol for 24 hours, the hyaline layer becomes greatly enlarged and well-defined in all parts of the organism. The area becomes more noticeable from day to day, and appears to be most noticeable on about the fourth or fifth day of exposure. The increase in depth of the hyaline layer is noticed in all concentrations from 0.05 *M* and higher. Another noticeable change is in the decrease in size of the pelomyxae in the high alcohol concentrations. After 8 days in 0.5 *M* alcohol, they are about 1/10 their original size. This is illustrated well in the figure.

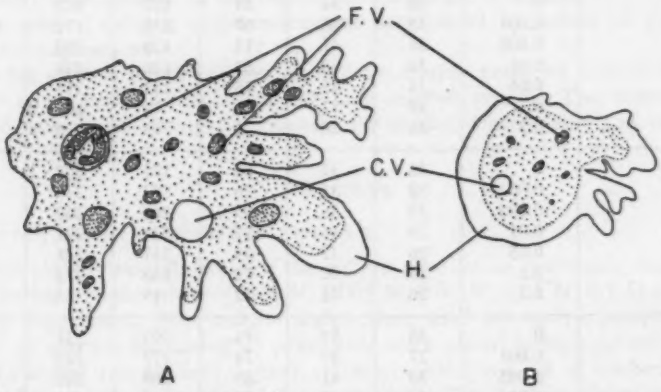

FIGURE 1. Camera lucida sketches of (A) a typical pelomyxa from a well-balanced culture solution without alcohol, and (B) one from the same solution containing alcohol in 0.5 *M* concentration. F. V., food vacuole; C. V., contractile vacuole; H., hyaline area.

The rate of digestion of food organisms is also retarded in high concentrations of alcohol. In the controls and in the 0.001 *M* to 0.01 *M* alcohol solutions, most of the food vacuoles contain only fragments of paramecia after 24 hours while in higher concentrations rather large fragments are found even after 5 days. In the 0.5 *M* alcohol solution the food vacuoles are carried over from the time of ingestion before exposure to alcohol; no paramecia are ingested by the pelomyxae after the latter are exposed to this concentration.

3. Effect of alcohol on respiration in Pelomyxa

The Barcroft-Warburg respirometer was used to ascertain oxygen consumption. This was done by means of the "direct method" as described by Pace and Belda (1944). Tests were made on pelomyxae in solutions without alcohol, and in solutions containing 0.005 *M* and 0.01 *M* alcohol. For each concentration, two sets of tests were run: in one, the organisms were introduced into the alcohol solution just

preceding the test; in the other, they were placed in the solutions 24 hours preceding the test. All the organisms were "well-fed" as described by Pace and Belda (1944).

To each Warburg flask, 500 pelomyxae were added. These were approximately uniform in size. The volumes of 30 pelomyxae selected at random were ascertained by means of a volumescope (Pace and Belda, 1944) before and after the tests and found to approximate 35,000 mm.^{3} per million organisms. The results are presented in Table II. The duration of each experiment varied between 5 and 9 hours. The oxygen consumption was ascertained in mm.^{3} per hour per million organisms as well as in mm.^{3} per hour per mm.^{3} protoplasm.

TABLE II

Oxygen consumption in Pelomyxa carolinensis in different concentrations of alcohol as compared to that in the absence of alcohol

Concentration of alcohol	Number of tests	Duration of experiment	Average rate of O_2 consumption in mm.^{3} per hr. per 10^6	Average O_2 consumption in mm.^{3} per hour per mm.^{3} cell substance
Organisms placed in test solutions just preceding test				
0 (control)	9	5–6 hrs.	9,800	0.280
0.005 M	9	5–6 hrs.	16,300	0.465
0 (control)	9	5–9 hrs.	7,200	0.205
0.01 *M*	9	5–9 hrs.	14,800	0.422
Organisms placed in solutions 24 hours preceding test				
0 (control)	9	5–7 hrs.	10,700	0.305
0.005 *M*	9	5–7 hrs.	23,500	0.671
0 (control)	9	5–7 hrs.	5,000	0.143
0.01 *M*	9	5–7 hrs.	20,900	0.568

Temperature, 25° ± 1° C.; average volume of 1 million pelomyxae, about 35,000 mm^3.

In both test solutions, the oxygen consumption is greater than in the control solutions which had no alcohol present. These results are therefore of a similar nature to those obtained in the growth studies in which growth increases appreciably in both these concentrations. In fact, the accelerating effect of alcohol is much more pronounced in the respiratory studies. The effect is even greater if the organisms are placed in the alcohol solutions for 24 hours before the tests are run.

DISCUSSION

Studies made upon ethyl alcohol and its effects on unicellular animals are not very extensive. In the earlier work cited previously, most of the tests were carried out with Paramecium as experimental animal although some observations were made upon amoeboid organisms. A comparison of the results seems to indicate that the amoeboid cells react to alcohol in a manner somewhat similar to paramecia which were usually killed by concentrations around 3% (0.75 *M*).

In the tests reported here, in which observations were made at various concentrations, most of the pelomyxae that had been put into 0.5 *M* (2.3%) alcohol solutions were dead at the end of 10 days; on the average, only 8 out of every 25 organisms were still alive.

In lower concentrations (0.005 *M* and 0.01 *M* alcohol) there was an actual increase in growth of these organisms. Associated with this growth increase is a very noticeable acceleration in oxygen consumption of the pelomyxae in the same concentrations of alcohol. This would suggest that the energy released on the oxidation of alcohol is available for certain processes in Pelomyxa.

Evidently much of the energy produced by this means is wasted; at least this is true if growth can be used as a measure for assimilative metabolism. For example, the optimum alcohol concentration for growth was found to be 0.005 *M*, in which there were produced on the average 281 organisms from 25 pelomyxae over a period of 10 days. This is a total increase of 256 organisms, and represents an increase of 30% over the control organisms without alcohol. On the other hand, in this same concentration of alcohol, there was a much higher percentage increase in oxygen consumption; a 66% increase in the organisms that were not adjusted and 119% in those that had lived in the alcohol for 24 hours before oxygen consumption was ascertained.

Summary

1. Specimens of *Pelomyxa carolinensis* were exposed to various concentrations of ethyl alcohol and observations made upon their rate of growth and respiration. The following concentrations were tested: 0, 0.001 *M*, 0.005 *M*, 0.01 *M*, 0.05 *M*, 0.1 *M*, and 0.5 *M* and 1.0 *M*.

2. In 1.0 *M* (4.6%) alcohol all the organisms were dead within 24 hours. In 0.5 *M* (2.3%), although most of them died early, some lived for a 10-day period.

3. Growth was accelerated in 0.005 *M*, 0.01 *M*, and 0.05 *M* alcohol; the greatest acceleration was a 30% increase over the control in 0.005 *M*.

4. In the higher concentrations of alcohol (0.5 *M* and 0.1 *M*) the pelomyxae do not feed and show considerable decrease in size; the hyaline layer becomes very large.

5. Rate of respiration was found to be much greater in 0.01 *M* and 0.005 *M* alcohol than in the controls without alcohol. It was greatest in 0.01 *M*, especially when the organisms were put into the alcohol solution for 24 hours before the tests were run, in which case respiration was 318% greater than in the controls.

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THE REGULATION OF SPONTANEOUS STRUCTURAL ANOMALIES IN PELMATOHYDRA OLIGACTIS

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It is necessary to specify the exact meaning of the word when employing the term regulation. Driesch (1901) defined it as a process or a change in a process occurring in a living organism, by means of which a disturbance of its pre-existing normal condition is wholly or in part compensated and the normal condition is re-established. Roux described regulation as a compensation of disturbance, *i.e.*, the return of the disturbed organism to a type or norm. Roux emphasized the part of heredity in the control of regulatory processes while Driesch was inclined to invoke extrabiological metaphysical controls. Morgan (1898), who had been working in the field of regeneration, coined the term morphallaxis to include the remodelling of old tissue into new forms. He later (1907) extended the use of the term to include re-differentiation and regeneration. Driesch used the terms as a synonym for restitution by redifferentiation. Morgan (1907) assumed that the influence of the formed parts upon the unformed parts during their development was a pressure or some kind of a response to a pressure. Child (1906) after working extensively on regeneration of Planaria and other forms came to the conclusion that living organisms are in a continual state of regulation physiologically and that no particular group of processes could be separated as formative as compared with those which, in the normal adult animal, maintained it in a state of partial equilibrium. Jennings (1905) applied the concept of regulation to the field of animal behavior and concluded that all animal behavior, in its more complex aspects as well as its simpler ones, is attributable to regulation.

Experimental embryologists point out (Weiss, 1939) that the course of embryology is rather fixed in animals with a mosaic type of development and that experiments involving interference with the regular course of development are not very productive. Other animals in which the course of development is not firmly fixed at an early stage, referred to as regulative types, will sometimes rearrange the cellular components in excised parts of an embryo and form one or more perfect embryos from material originally destined to form only a part of an embryo. Spontaneous abnormalities in embryos may rearrange their parts so as to form a normal embryo. The term regulation is used to indicate the processes by which the embryos undergoing experimental interference or embryos having spontaneous anomalies rearrange their materials so as to produce perfect whole individuals. It is assumed that the processes and the controls of the processes are identical with those which guide development in the course of normal development. Presumably the regulative mechanism continues to exist in fully formed individuals although, under normal conditions, there is no opportunity for the mechanism to be exhibited.

Curtis defines regulation as all of the processes by which the organism returns to the physiological and morphological type that characterizes the species after the

type has been disturbed. Regeneration is considered to be a form or phase of regulation. Morphallaxis is defined as the process by which proportions are regained after disturbance.

Needham (1950) confines the term morphallaxis to morphological modification or regeneration without the formation of new cells. Regulation is described as the power of continuing normal or approximately normal development or regeneration in spite of experimental interference. The processes operate in all normal embroys and are a part of the sum total of processes whereby the organism is to some extent rendered independent of its environment.

This survey of the uses of the terms regulation and morphallaxis indicates that there are considerable differences in the interpretations of their meanings. The writer will use the term regulation in much the same sense as it is employed by Child (1924). He points out that in the reconstitution of a whole from a part during regeneration, the course of development in a reconstituted piece of an animal is not normal (as compared with the course of development which produced it in the first place) but the results as regards form and structure are normal. The over-all result of regulation will be regarded as a kind of holding action by which an aberrant specimen is returned to the parental type. The term regulation will be used strictly, as it is by Hyman (1928), for the series of spontaneous changes by which an anomalous specimen is returned to the normal species and clonal type. None of the anomalies to be described arose because of experimental interference.

No attempt will be made to determine the character of the regulative mechanism but it will be re-emphasized that the regulative course through which morphological anomalies are returned to normal types is very different from that through which the normal type was produced in the first place. The regulative processes occur in the presence of such mature states as budding, depression, formation of sexual organs, ect. The influences of these states upon regulation will be given particular attention.

Material and Methods

The specimens of *Pelmatohydra oligactis* used in this study were all the progeny of a single male specimen with 5 tentacles. Some of the observations on regulation were incidental to a study of the daily rate of reproduction and the reproductive potential of a single clone (Turner, 1950). In most of the cases of aberrancy in structure, the individuals concerned were members of a pedigreed strain which had been under observation for many months and for which a complete record had been kept. A large discard pool for the clone was kept in a 50-gallon tank. Specimens which were not studied in individual cultures were placed in this pool and given maximal feeding. Thousands of specimens were produced in the pool and the pool was examined from time to time for aberrant specimens. When aberrant specimens were discovered they were removed from the pool and cultured individually so that continuous individual records could be kept.

Records of pedigreed specimens as well as specimens selected and cultured from the pool consisted of notations on tentacle number, formation of spermaries, number and rate of bud production, duration of processes concerned in regulation (absorption of parts, fusions, divisions, migration of specific parts, etc.), and the cycles of depression and recovery.

Longitudinal Fission

There are a number of temporary conditions which, when considered in a single stage, give the appearance of longitudinal fission. It is necessary, therefore, to follow suspected cases through to a conclusion before they can be classified definitely as cases of apico-basal fission. All the eight cases described here occurred in a single clone, in either pedigreed stock or in a discard pool of the clone. All were put under daily observation and records were kept until fission was complete or the specimens died. Five of the cases were found in specimens of which daily records had been kept and three were discovered in the discard pool. The complete records have made it possible to determine the total length of time occupied in fission and the relative lengths of time required for fission of the different parts of the body. The relation of fission to spermary formation and to periods of active budding and depressions was also observed.

All of the five cases which originated in pedigreed stock under observation began in buds which had not yet become detached. In all of these cases the parent animals had just emerged from a state of depression and the attached buds had gone through the depression with the parents. One of the three cases discovered in the discard pool was in an incipient stage of development as indicated by the half-developed tentacles. No instance was observed in old fully developed specimens in which a bifurcated condition of the apex resulted later in longitudinal fission. Numerous cases of a bifurcated apex in old specimens have been observed, but the return to a normal state was attained by other methods of regulation.

Two of the cases of longitudinal fission were recorded as follows:

Specimen 1. The specimen was observed as a partially developed bud with a bifurcated apex. Development was completed by the next day when the bud separated from the parent. Six days later a bud appeared upon the newly formed bifurcated individual at a point well below the region of the bifurcation. Fission was slow, requiring 38 days to reach the budding zone. During the 38-day period 21 buds were given off. The fission of the single budding zone required five days during which one of the apical members gave off seven buds and the other gave off eight buds. Eight days were required for the division of the stalk below the budding zone and of the base. During the last eight days of fission the specimen was in a state of depression during which it did not feed and gave off no buds.

Specimen 2. This specimen was observed first as a bifurcated bud which was about to be separated from the parent. After separation from the parent, which occurred 16 hours after the first observation, the specimen began budding for itself. The first bud appeared just below the point of bifurcation three days after separation from the parent. By the end of 33 days fission had reached the budding zone and eight days were required for the division of the budding zone. The stalk below the budding zone and the base was completely divided in another nine days. A total of 19 buds was given off before fission reached the budding zone. During the division of the budding zone, the stalk and the base, nine buds were given off by one member and 11 buds by the other. Spermaries formed upon the undivided and also the divided portion of the specimen 17 days after it had separated from the parent and persisted for seven days. During the period of observation two

depressions occurred, one lasting for four and the other for six days. The state of depression appeared to have no effect upon the progress of the process of fission. Fission of the stalk and of the base was completed during a period of active budding.

There was some variation in the division time of the other three specimens recorded from pedigreed stock and in the three specimens discovered in the discard pool. The time required for the first appearance of buds on bifurcated specimens after they had separated from their parents varied from three to seven days. Division from the hypostome down to the budding zone required from 27 to 41 days. The time involved in the division of the budding zone was brief and varied from 5 to 11 days. The division of the stalk was relatively rapid and in no case required more than six days. Fission lagged upon reaching the base and in the most extreme case 12 days were required for its complete division.

One aberrant specimen should be described here. The specimen separated from the parent as a bud which was almost completely developed. The apical end was bifurcated but one hypostome was larger and better developed than the other. The specimen fed for one day only, through the better developed mouth and hypostome, and then went into a prolonged depression from which it did not recover. It became inactive, the tentacles became short and bulbous and there was no formation of buds or sexual bodies. However, the process of longitudinal fission continued and was completed seven days after separation from the parent. Fission resulted in the formation of two individuals of unequal size, both of which disintegrated two days after fission was complete.

Two buds which had deep divisions in the hypostomal regions were permitted to separate from the parent animals and they were then submitted to complete starvation. Both of the specimens died in less than a month and during this time longitudinal division did not progress.

Chang, Hsieh and Liu (1952) found that fission occurred in a ratio of about 1/1000 to normal buds. They found that buds with twin apical ends might arise either through division or by fusion of specimens located in close proximity on the parental stock. They noted the surprising situation also of complete division of buds before detachment from the parent. Neither of these phenomena were observed in the stock used by the writer.

Summary: A summary of the facts relating to longitudinal fission indicate that: (1) the bifurcated condition of the apex which initiates longitudinal fission originates most commonly, and possibly exclusively, in buds which are in the latter stages of development and are still attached to the parent. A bifurcated condition of the apex is fairly common in mature specimens recovering from a physiological depression but none of the specimens observed in this condition resolved the abnormality by undergoing longitudinal fission. (2) In most cases of longitudinal fission the hypostome had been divided previously into two equal parts to initiate fission. In exceptional cases hypostomes which had been divided unequally initiated complete fission. (3) The early phases of fission involving division down to the budding zone are accomplished in a relatively short time (5 to 11 days). (4) The division of the stalk and the base requires about eight days ordinarily but one specimen required only four days and another 16 days. (5) The progress of longitudinal fission does not seem to be retarded by a state of depression in a specimen. (6) A state of active budding does not appear to affect the rate of fission. (7)

Spermaries may be formed, come to maturity and disappear without changing the rate of fission. (8) Complete starvation appears to stop the progress of longitudinal fission.

Reduction of Supernumerary Hypostomes and Tentacles

Sixteen specimens were observed among approximately 3500 pedigreed individuals of a single clone in which at some period the hypostome was divided and reduced to the single state again by fusion. While this appears to represent a high incidence for the condition, it is believed, for reasons that will be mentioned later, that cases in which the hypostome undergoes slight division and subsequent fusion are quite common. Two of the 16 cases observed occurred in buds which separated from the parents with slight but clear cut divisions of the hypostome, two mouths and supernumerary tentacles and later regulated into single individuals by fusion of the hypostomes and tentacles. In the two cases occurring in buds the divided hypostomes were composed of unequal parts. The remaining 14 cases occurred among old specimens. All of the cases were observed for several weeks after their discovery. A search of the discard pool of the same clone revealed other specimens with divided hypostomes but these were not studied further.

All of the instances of divided hypostomes in old individuals occurred in specimens which, after a period of reproduction by budding, had gone into a state of depression characterized by inactivity, cessation of budding and feeding and a partial disintegration of the hypostome and tentacles and were recovering from the depression. Periods of depression were of two types, one lasting from one to four days and the other from 6 to 12 days. The most pronounced disintegration occurred in specimens undergoing the longer periods of depression. The divided hypostomes occurred in specimens which were regenerating the hypostomes after depression and partial disintegration. In every case, supernumerary tentacles appeared. When the hypostome was divided equally and two functioning mouths were present the number of tentacles was doubled so that 10 tentacles were present. When the hypostome was unequally divided and two mouths were present but only one was functional, the number of supernumerary tentacles was less. It would appear that the condition of a divided hypostome should result in longitudinal fission but no such fission was observed in these old specimens. Instead, a fusion occurred which reduced the doubled condition of the mouth and hypostome to a normal condition within a few days. The reduction of the supernumerary tentacles by fusion to the 5 characteristic of the clone took much longer.

In rare instances, divided hypostomes in buds became fused instead of initiating longitudinal fission. A bud with a divided hypostome was discovered on an old individual bearing three other buds. The parental individual was in the midst of an accelerated reproductive period. The partially divided bud was second from the oldest of the attached buds and since no period of depression had occurred for several days, it is obvious that the bifurcated condition of the bud, unlike that in mature individuals, did not arise because of a distributed growth rate produced by a depression and a recovery from the depression. The buds just older and younger than the partially divided one were normal in every way and it is not possible reasonably to assign any recognized state of the bud or of the parent as the cause of the bifurcation. The partially divided individual was examined daily for 52

days after it was detached from the parent. The divided hypostome was fusing before the bud became detached and it was completely fused within three days of detachment. Three short supernumerary tentacles, which had arisen while the hypostome was divided, were irregularly disposed on the hypostome, two outside the regular ring of 5 and one inside the ring near the mouth. This small tentacle near the mouth was absorbed within two days. The other supernumerary tentacles were completely absorbed within 18 days without fusing with any of the tentacles in the regular ring of 5. The specimen gave rise to 34 buds during the next 52 days, each bud bearing 4 or 5 tentacles when detached and 5 tentacles when mature.

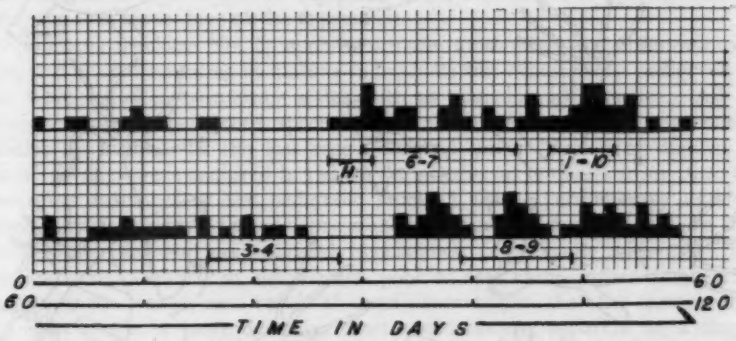

DIAGRAM 1. Block diagram showing relation of reproduction by budding and of depressions in Specimen 5.1.10 to regulatory processes. Blocks represent new buds produced per day. H represents period of fusion of divided hypostome; 6–7, 1–10, 3–4, 8–9 represent periods of fusion of tentacles indicated by the numbers.

The fairly typical formation of a divided hypostome and the subsequent regulation of the aberrant condition by fusion of the hypostome and tentacle in a mature specimen is shown in Diagram 1 and in Figures 12 to 18. In Diagram 1 blocks represent the number of new buds formed per day. The largest number of buds formed in one day is four. It will be noted that periods of rapid bud production are interspersed with periods of one to three days during which specimens produce no buds. Two prolonged periods of depression occur between the 18th and 27th day and the 85th and 93rd day. The four-day period of time indicated by H is the period during which the divided hypostome fused and the periods of time indicated by 6–7, 1–10, 3–4, and 8–9 are those during which the numbered tentacles were fusing. The 120 day history of the specimen is as follows: During the first 17 days of observation, 10 buds were produced with intervals of rest of two to three days between periods of budding. The parent animal had 5 tentacles (Fig. 12) as did the buds. A period of depression occurred on the 17th day (Diag. 1), during which the mouth disappeared, part of the hypostome disintegrated and the tentacles were reduced to short stubs (Fig. 13). The period of depression was followed by rapid recovery involving regeneration. During regeneration (Diag. 1, day 23 to 26) two hypostomes and two mouths were formed. Nine tentacles of the usual length were formed and one short tentacle (Fig. 14, 1). Fusion of the two hypostomes (Figs. 14, 15) occupied four days (Diag 1). Before fusion of the

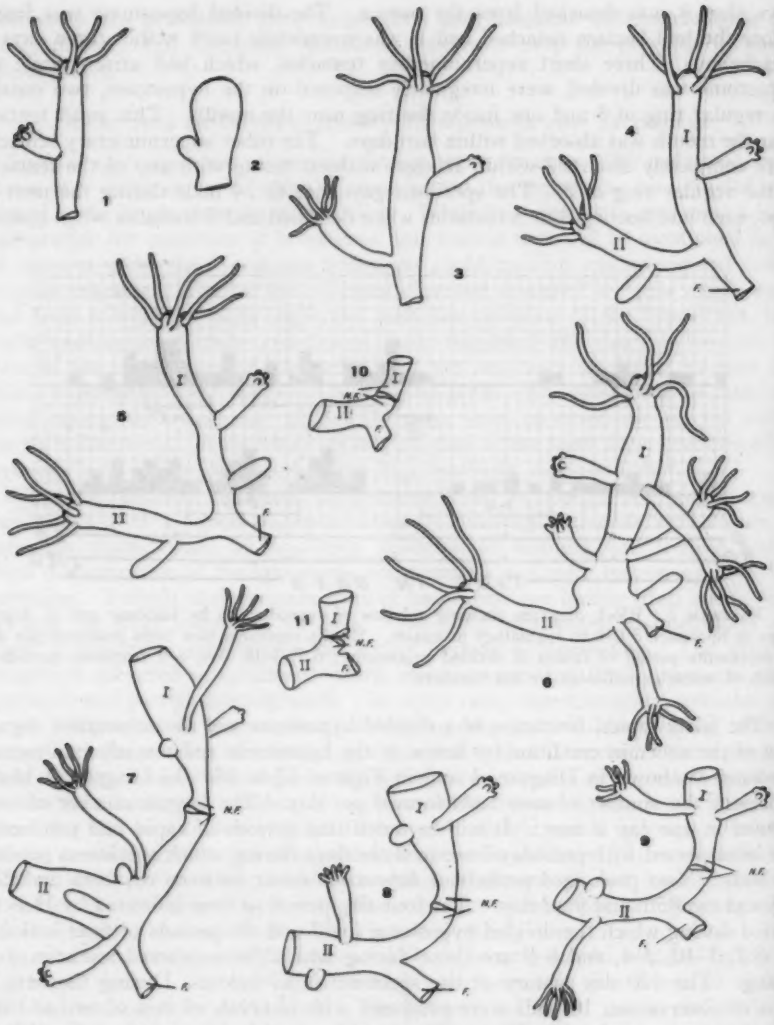

Plate 1

FIGURE 1. Specimen of male *Pelmatohydra oligactis* with attached bud prior to period of depression. Following figures indicate progress in the same specimen.

FIGURE 2. The specimen during depression.

FIGURE 3. Emergence from depression; regeneration of apical parts by parent and bud; shifting of bud toward base of parent; resumption of budding by parent.

FIGURE 4. Failure of old bud to detach (II); old bud producing bud of its own.

FIGURE 5. Budding zone of parent (I) elongating; both parent (I) and old retained bud (II) budding rapidly to form colony.

hypostome was complete, the specimen was budding vigorously (Diag. 1, days 27–35) and tentacles no. 6 and 7 were fusing, beginning at the base and proceeding apically. The fusion required 14 days. A fusion of tentacles 1 and 10 began on the 47th day and was completed by the 53rd day (Fig. 16) (Diag. 1, 1–10). Figure 17 represents the specimen on the 75th day. Tentacles 3 and 4 fused between the 76th and the 88th day (Diag. 1, 3–4) (Fig. 18) and tentacles 8 and 9 fused between the 99th and the 109th day (Diag. 1, 8–9). A typical long depression and a recovery occurred between the 85th and the 93rd days (Diag. 1) but no abnormalities arose in connection with this period. Tentacles 3 and 4, which had been fusing for 9 days before the beginning of the depression, completed their fusion during the first three days of the depression.

Specimens in clone No. 5, upon which the most extensive observations were made, had 5 or 6 tentacles in mature individuals but occasionally pedigreed specimens were found in temporary states with as many as 10 tentacles. All of the cases occurring in pedigreed specimens reduced the number of supernumerary tentacles by fusion. In the last case described under "fusion of divided hypostomes" 80 days were required to reduce the number of tentacles from 10 to 6. In some other instances in the pedigreed stock, a few supernumerary and poorly formed tentacles appeared after depressions and recovery from depressions in individuals which did not have divided hypostomes. Specimens with more than 5 tentacles were observed in the discard pool of this clone rather frequently. It occurred to the writer, as a result of these observations, that the tentacle number for a clone might be more definitive than would become apparent by a single examination of a large number of individuals. A single examination would indicate a considerable range in number and it would be reasonable to assume this to be the natural range of variation. However, if it is a fact that a narrow definitive range of tentacle number exists in a clone, then all of the specimens with a tentacle number larger than the definitive number could be in a state of regulation during which the definitive tentacle number would be restored.

The hypothesis that large numbers of hydra are normally in a state of regulation as regards tentacle numbers and that nearly all specimens with supernumerary tentacles should be included among the regulating specimens can be tested by several different types of observations. (1) The tentacle number can be recorded for all of the buds of a single parent animal which itself is the product of a line with a definitive number of tentacles. The production only of buds with the same number of tentacles as the parent would indicate a maintenance within the clone of the definitive number. (2) Departures by buds from the tentacle number characteristic of the parent can be associated with events, if any, in the life history of the clone producing the bud. (3) Discard pools of clones with a definite number

FIGURE 6. Appearance of transverse diaphragm (dotted line) in the budding zone of parent (I); fusing and adjustment of budding zones of individuals I and II.

FIGURE 7. Apical parts not shown; further progress of tranverse division and differentiation of new feet (N.F.) at site of diaphragm.

FIGURE 8. Apical parts not shown; migration of region of old foot (F.) toward site of transverse division (N.F.).

FIGURE 9. Further migration of old foot; both individuals (I and II) budding actively.

FIGURES 10 and 11. Completion of migration of old foot to fuse with nearest of new feet; budding is active but bases only are shown.

of tentacles can be examined from time to time and if specimens with supernumerary tentacles appear they can be isolated and observed. (4) A census of tentacle number can be taken of all the specimens in a discard pool from time to time to determine whether the total range of variation has changed. (5) Specimens with tentacle numbers larger than ordinary, from sources other than discard pools, can be isolated and observed. All of these types of observations have been used and the results have supported the hypothesis that the degree of variation in tentacle number is not great and that the specimens which vary much from the clonal number are in a state of regulation.

The most complete record of a specimen which reduced the tentacle number by fusion has been described (Figs. 12 to 18; Diag. 1) under the section on "fusion of divided hypostomes." Descriptions of some other cases offering a number of variations follow.

Specimen 5.1.3 from a clone with a characteristic tentacle number of 5 had 7 tentacles after a two day depression and a one day recovery. The specimen had previously given rise to 107 buds at the approximate rate of 1.14 buds per day. During the short period of depression the hypostome degenerated and the tentacles became short and bulbous. On recovery, there were 5 tentacles in the normal position and two short tentacles on the hypostome within the ring of 5. As all of the tentacles increased in length, the short tentacles began fusing at the bases with the nearest tentacles within the ring and within five days the fusion was complete.

Specimen with 7 tentacles. This specimen arose from a bud with a divided hypostome in non-pedigreed stock. After the bud separated from the parent the divided hypostome fused into a single one with 8 tentacles of irregular lengths placed around the hypostome in an irregular row. The shortest tentacle was absorbed by the hypostome within two days without fusing with any other tentacles and the remaining 7 tentacles became adjusted so as to form a perfect ring about the hypostome. The 7 tentacles became equalized in length by differential growth. The specimen lived for 17 days during which it gave off 31 buds, 25 of the buds having 5 tentacles and 11 buds having 4 tentacles. These latter individuals developed 5 tentacles when mature.

Specimen with 9 tentacles. This pedigreed individual on recovery from a depression had 9 tentacles. Two tentacles were fused (or forked) near the ends, two were located upon the hypostome near the mouth and five were normal and in normal positions. The forked pair became single by fusion within 24 hours. The two short centrally placed tentacles first fused with each other and were then ab-

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FIGURE 12. Specimen 5.1.10 in normal active condition. All figures are of the same specimen.

FIGURE 13. Partial disintegration during period of physiological depression.

FIGURE 14. Condition following depression and recovery; two mouths, two hypostomes and supernumerary tentacles.

FIGURE 15. Fusion of two hypostomes in progress; tentacles no. 6 and no. 7 fusing.

FIGURE 16. Two hypostomes are completely fused; tentacles 6 and 7 are completely fused; tentacles 1 and 10 are in process of fusing (22 days after recovery from depression).

FIGURE 17. Fusion of tentacles 1 and 10 complete.

FIGURE 18. (53 days after recovery from depression). Tentacles 3 and 4 are fusing. (Tentacles 8 and 9 began to fuse 72 days after recovery from depression and were completely fused 11 days later.)

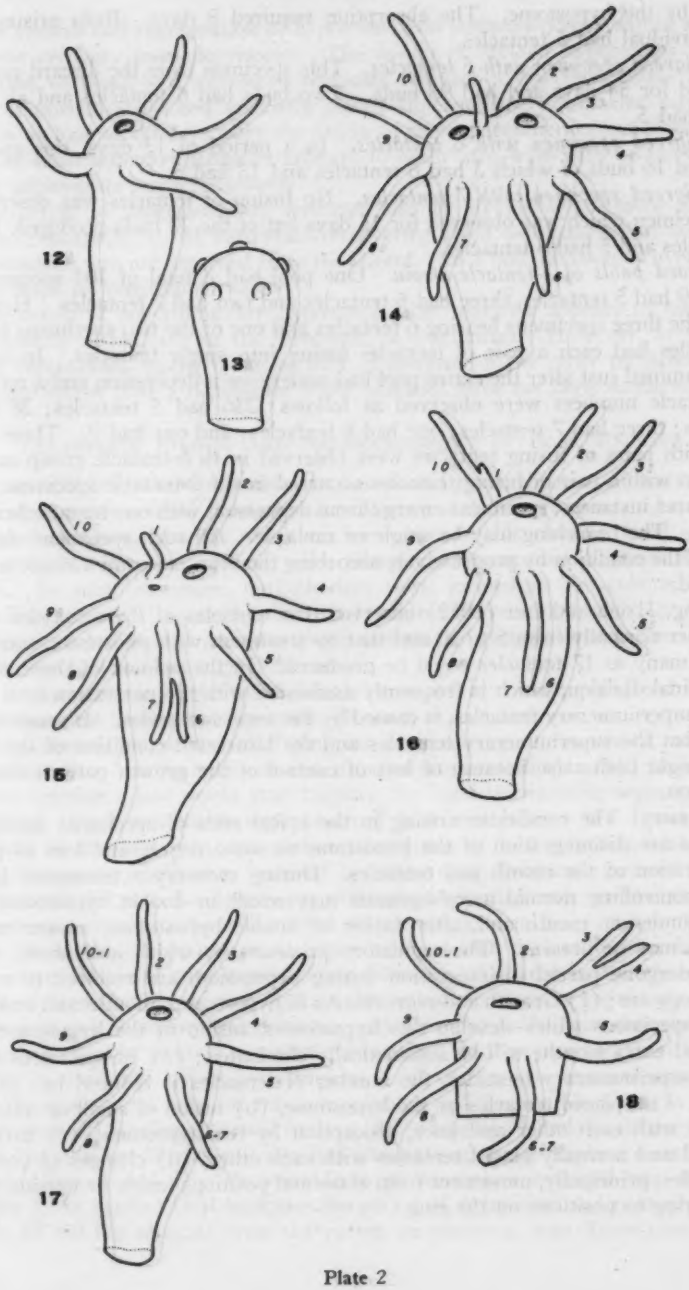

Plate 2

sorbed by the hypostome. The absorption required 8 days. Buds arising from this individual had 5 tentacles.

Pedigreed specimen with 5 tentacles. This specimen from the discard pool was observed for 54 days and had 96 buds. Two buds had 6 tentacles and all of the others had 5.

Pedigreed specimen with 6 tentacles. In a period of 13 days, this specimen produced 16 buds of which 3 had 6 tentacles and 13 had 5.

Pedigreed specimen with 7 tentacles. No fusion of tentacles was observed in this specimen which was observed for 15 days but of the 20 buds produced, 15 had 5 tentacles and 5 had 6 tentacles.

Discard pools of 5-tentacle strain. One pool had a total of 104 specimens of which 99 had 5 tentacles, three had 6 tentacles and two had 7 tentacles. However, one of the three specimens bearing 6 tentacles and one of the two specimens bearing 7 tentacles had each a pair of tentacles fusing into single tentacles. In another pool examined just after the entire pool had undergone a depression and a recovery, the tentacle numbers were observed as follows: 236 had 5 tentacles; 38 had 6 tentacles; three had 7 tentacles; one had 8 tentacles; and one had 9. Three specimens with pairs of fusing tentacles were observed in th 6-tentacle group and one specimen with a pair of fusing tentacles occurred in the 9-tentacle specimen.

In rarer instances, specimens emerge from depression with one tentacle branched or split. The branching may be single or multiple. All such specimens observed resolved the condition by progressively absorbing the branches until a single tentacle remained.

Chang, Hsieh and Liu (1952) observed that tentacles of *Pelmatohydra* varied in number normally from 5 to 10 and that by treatment with phosphate, specimens with as many as 17 tentacles could be produced. In the opinion of these authors longitudinal division, which is frequently associated with the possession by a specimen of supernumerary tentacles, is caused by the excess tentacles. It seems to this writer that the supernumerary tentacles and the bifurcated condition of the hypostome might both arise because of loss of control of the growth pattern during a depression.

Summary: The conditions arising in the apical ends of specimens during depressions are disintegration of the hypostome to some degree and loss of partial disintegration of the mouth and tentacles. During recovery a temporary loss of factors controlling normal morphogenesis may result in double hypostomes with a supernumerary mouth and, after fusion of double hypostomes, supernumerary tentacles may be present. The regulatory processes by which individuals, which have undergone partial disintegration during depression, are restored to normal morphology are: (1) Growth and regeneration of hypostomes, mouths and tentacles; (2) In specimens which develop two hypostomes, fusion of the hypostomes will occur and extra mouths will be automatically eliminated; (3) In specimens which possess supernumerary tentacles, the number of tentacles is reduced by: (a) absorption of misplaced tentacles by the hypostome, (b) fusion of small or misplaced tentacles with each other and later, absorption by the hypostome, (c) fusion of full sized and normally placed tentacles with each other, (d) changes of positions of tentacles, principally, movement from abnormal positions inside or outside of the regular ring to positions on the ring.

The growth and regeneration of hypostome and tentacles occurs within a day or two after recovery from depression. The fusion of divided hypostomes and the absorption of small and misplaced tentacles requires a few days more and the fusion of normal sized and normally placed supernumerary tentacles may be in progress as long as 80 days after the origin of the supernumerary condition. The fusion of supernumerary tentacles appears to occur step-wise with no overlapping in time of separate tentacle fusions.

The origin of supernumerary tentacles and of divided hypostomes has been observed repeatedly in old individuals recovering from depressions but only once in a bud which was not detached from the parent. In a bud, division of hypostomes usually leads to longitudinal fission.

The fusion of supernumerary hypostomes and tentacles appears to take place independently of budding or the formation of spermaries. However, in the case of long depressions, fusing tentacles may disintegrate if the other tentacles do so, or resume fusion after a depression if the tentacles have not been damaged.

Transverse Fission and Rearrangement of Body Regions

Hyman (1928) observed transverse fission and rearrangement of body regions in four male specimens of *Pelmatohydra oligactis* and their progeny. As a result of heavy feeding or, in some instances, for unaccountable reasons, buds were retained to form temporary colonies after changing their normal positions upon the parents. In some instances, multiple feet were formed in the colonies. Final results indicated that transverse divisions occurred in positions which divided the colonies into equal masses and that single normal individuals were formed eventually with one foot and one hypostome each.

In the present study, four individuals occurred in a male pedigreed stock in which transverse fission was observed. In each case, a bud was retained upon the body of a parent and, after establishing its independence, it functioned as a separate individual. A temporary colonial situation arose often in rapidly budding individuals because a bud would start budding for itself before being separated from the parent. Such conditions were resolved in a matter of a few hours by the separation of the bud from the parent. Instances of bud retention resulting in colony formation, which was resolved by transverse divisions, occurred only in specimens which entered a depression with a bud attached and, on emerging from the depression, retained the bud instead of separating from it. Once the bud had passed the period for separation, it began to reproduce by budding for itself and at the same time the parent was doing likewise. Entirely independent of the process of reproduction in each part of the two-part colony, a regulatory process involving the whole colony was occurring which tended to separate the whole mass into two equal parts, to form new parts where they were required for normal structure and to shift and adjust body regions. All of the four cases observed were somewhat similar and only the most complicated will be described.

Specimen 5.3.3 under maximal feeding had produced 31 buds in 28 days (Diag. 2) and bud number 32 was still attached when the specimen entered a state of depression (Figs. 1 and 2). Recovery from its depression required two days. In Diagram 2, the symbol D–R indicates the period of depression and recovery. Bud number 32 did not separate from the parent on recovery from depression but re-

mained attached and shifted its position nearer to the base of the parent (Fig. 3). Active budding was resumed by the parent and 48 buds were produced in the next 31 days (Diag. 2, I). In the same period of time the retained bud produced 31 buds of its own (Diag. 2, II). Within a few days after resumption of budding by the parent (Fig. 5, I), the budding region began to elongate as indicated by the longer distance between the upper and the lower buds. Two days later, a delicate transverse diaphragm appeared within this elongated budding zone, separating buds in the lower budding zone from the upper budding zone (Fig. 6). The diaphragm marked the beginning of a transverse division and the site in which two new feet

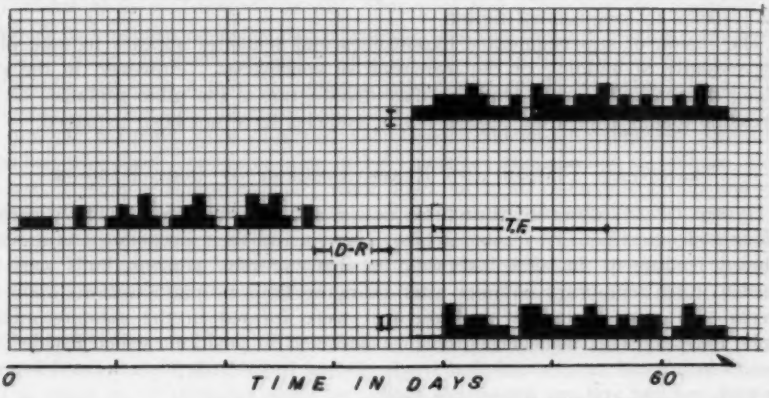

DIAGRAM 2. Block diagram showing relation of reproduction by budding to transverse fission in Specimen 5.3.3. Blocks represent number of new buds produced per day. D-R indicates period of depression and recovery in parent. I represents continuation of that portion of parent above site of transverse division; II represents retained bud and that part of the parent taken over by the retained bud. T.F. represents period from first appearance of transverse diaphragm to the end of fission.

were to be formed. In Figure 7, it is indicated clearly that the parent individual (I) had become re-oriented, at least in its budding zone, upon the new foot (N.F.) while individual II had its budding zone oriented upon both the old foot (F.) and the new foot region (N.F.). The differentiation of the new feet, and the separation of the two individuals I and II required 16 days (Diag. 2, T.F.) (Figs. 6–11). It is indicated in Figure 8 that both individuals I and II had become oriented upon the new foot region although the only functional foot for the colony was the old foot (F.). Before the individuals became completely separated and the new feet became functional, the position of the old foot changed progressively (Figs. 8, 9, 10, 11, F.) until the foot became fused with the nearest of the new feet so that in spite of the formation of two new feet in a new position, the actual locus of the attachment of the animal to the substratum remained always the same. The actual separation of individuals I and II had no influence upon the continuity or rate of budding in the two individuals. The termination of the process of transverse fission shown by the right end of the line T.F. in Diagram 2 did not disturb bud production.

Summary: In the cases observed in this study, transverse fission occurred in temporary colonies produced by retention of a bud by individuals which had just recovered from a depression. In due time, a transverse division occurred in such a position as to divide the colony into two equal masses. New feet arose at the site of the transverse division and re-orientation of body regions, particularly budding zones and new and old feet, occurred so as to produce single normal specimens. No cases of transverse division were observed except in connection with the resolution of temporary colony formation.

Discussion

There is little reason for assuming that transverse and longitudinal fissions are methods of reproduction. It is a fact that fissions result in the formation of additional individuals but it is true also that the fissions assist in resolving abnormalities of form and in restoring normal structure. The opinion of the writer agrees with that of Hyman (1928) (p. 73) "that fission in hydra is not a normal method of asexual reproduction but a mode of regulation of previously existing abnormalities."

The capacity for regulation in *Pelmatohydra* is very extensive and the total capacity is studied better by experiments involving extirpation of parts, coalescence, grafting, etc. Reversals of polarity, regeneration, rearrangements of body parts and other regulatory features may be demonstrated easily. In this paper interest is centered not so much upon regulative capacity as spontaneous regulation in relation to the events of the normal life history of *Pelmatohydra*. Although the types of regulatory processes described by Hyman (1928) and by the writer are rather few it is assumed that other types of spontaneous regulation may occur under appropriate natural conditions.

In some animals (other than hydra) the adults seem to have retained little or no capacity for regulation. In some others regulation appears only spasmodically in the repair of tissues injured by accident or in the correction of structural anomalies arising after accidental injury. Regulation in these cases appears as an extraordinary rather than a normal process. Regulation in *Pelmatohydra* is seen as an almost continuous process because of the frequency of the origin of structural deviations from the clonal type, particularly in tentacle number, and the persistent tendency to correct these deviations even when they have no functional significance. The sequence of events in which regulation plays a part under optimal conditions as found in nature or in the laboratory is roughly as follows: (1) There is a period of reproduction during which buds are produced rapidly, as rapidly as 1.14 buds per day. (2) Each period of rapid reproduction is followed by a period of depression during which control of growth patterns is partially or wholly lost. (3) Growth and regeneration when resumed may give rise to atypical structures. (4) Regulation corrects the atypical character of the structures so that the clonal type of structure is restored. This sequence of events can hardly be considered abnormal for *Pelmatohydra*. Reproduction and depression appear whenever optimal conditions for budding occur. Some phase of atypical form arises frequently and as surely as it arises, regulation follows. In the animals possessing little capacity for regulation, atypical structures would become permanent if they did not

interfere with vital processes and they could prevent survival of an animal if they affected important organs. *Pelmatohydra* happens to be a form which has retained great regulative capacity even in the adult and since the formation of atypical structures in the sequence of events indicated above is common, a balancing regulative process is also common.

There has been a tendency in the literature on regulation to emphasize a unitary control for normal morphogenetic processes and regulation. During the embryology of animals with a regulative type of development the forms of embryos can be experimentally altered but on recovery a normal development is re-established. An assumption of unitary control for development both before and after experimental interference with the embryo is logical. However, the assumption of unitary control for both embryonic development and for regulation in *Pelmatohydra* does not lead to clarity. Rather, it seems that the difference between normal morphogenesis and regulation is the conspicuous feature. In embryonic development as well as morphogenesis initiated by budding, the processes are characterized first by cell proliferation, then growth and finally by differentiation. The development is very rapid in buds. The final result is an animal of rather constant form with a specific number of tentacles. In regulation, on the other hand, the changes occur in a fully matured and differentiated animal with atypical structures and the processes which restore the atypical animal to normal form do not occur in embryology. Some of the processes concerned in regulation occur rapidly, *i.e.*, absorption of supernumerary hypostomes and of supernumerary tentacles. Some other processes such as apico-basal division and transverse fission require more time and in one case described above in which there was a persistent reduction of supernumerary tentacles (from 10 to 6) the regulative tendency lasted for 110 days. The existence of a dual set of controls in regulating animals is best seen in those which undergo profuse budding during the regulatory process. In these cases the animal with supernumerary tentacles is giving rise rapidly to numerous buds which bear tentacles in the number characteristic of the clone. At the same time the same animal is slowly reducing the number of its own tentacles by fusion to approximate the number characteristic of the clone. The same end, normal adult structure, is being attained by different processes proceeding in different directions at different rates.

Further evidence for independent control of regulation is seen in the fact that regulation occurs equally well in specimens which are budding profusely or in specimens which are in temporary depression and are not budding. Waves of spermaries arise, reach maximal stages and then wane without affecting the persistent tendency toward regulation in specimens with atypical structure.

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PHYSIOLOGY OF INSECT DIAPAUSE. IV. THE BRAIN AND PROTHORACIC GLANDS AS AN ENDOCRINE SYSTEM IN THE CECROPIA SILKWORM

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In the previous papers of this series (Williams, 1946b, 1947, 1948a) an endocrine basis was described for the production and termination of pupal diapause in the Cecropia silkworm. The onset of diapause was correlated with a temporary failure of the brain in secreting a hormone required for the initiation of adult development. The ultimate release of this "brain hormone" was found to terminate dormancy and to set in motion the process of adult development. The brain was therefore viewed as the organ of primary control over the genesis and termination of diapause in the Cecropia silkworm.

After more detailed examination it became apparent that the brain hormone is fundamentally a tropic factor whose primary target within the diapausing pupa is a second endocrine tissue, the prothoracic glands. The latter, under stimulation of the brain hormone, then secrete the factor reacting with the pupal tissues to terminate diapause (Williams, 1947–1951). The brain and prothoracic glands were therefore considered to function as an endocrine system in controlling the pupal diapause. However, the role of the brain hormone was not fully clarified in the above-mentioned studies. There remained the possibility that the brain hormone might also act directly on the pupal tissues to condition their ultimate response to the prothoracic gland hormone.

The roles of the brain and prothoracic glands have therefore been examined in further detail in the present investigation. Attention has also been focussed on the possibility that, at earlier periods in the life history, the same hormonal system may control the moulting and pupation of the larval insect.

Materials and Methods

The present report is based on a study of approximately two thousand Cecropia silkworms (*Platysamia cecropia*). In certain experiments other Lepidoptera, including *Telea polyphemus, Actias luna, Actias selene, Antheraea mylitta, Bombyx mori, Danaus plexippus, Lymantria dispar* and *Prodenia eridania,* were used as donors and recipients of various endocrine organs. The insects were reared in large numbers from eggs obtained from fertile females. In general, the management of the experimental animals was essentially the same as that described previously (Williams, 1946b).

[1] This study was aided by the Lalor Foundation, by a research grant from the U. S. Public Health Service, and by an Institutional Grant to Harvard University from the American Cancer Society, Inc.

Carbon dioxide anesthesia was used in all surgical procedures (Williams, 1946a). In experiments performed on pupae, surgical mortality was minimized by implanting several crystals of phenylthiourea and/or a few drops of cyanide Ringer's solution.[2] By temporarily blocking the enzyme tyrosinase, these agents served to protect postoperative pupae from toxic quinone intermediates which appear in the blood if the latter is permitted to darken as a result of the tyrosine-tyrosinase reaction. In certain experiments, a few crystals of streptomycin sulfate and potassium penicillin G were also implanted. These antibiotics virtually eliminated bacterial infection which otherwise complicates certain experiments, especially those performed on isolated pupal abdomens; their use was found to introduce no discernible complications.

The Prothoracic Glands as the Source of the Hormone Terminating Pupal Diapause

In order to induce the adult development of an isolated abdomen it is necessary to implant a brain from a previously chilled pupa, plus prothoracic glands obtained from either chilled or unchilled pupae (Williams, 1947). If the brain functions solely by activating the prothoracic glands, then it should be possible to accomplish this same end in the absence of the brain by implanting prothoracic glands which have already been activated by the brain hormone. Moreover, according to results reported previously (Williams, 1947), the glands should exist in this functional state when obtained from post-diapausing pupae during the first five days of adult development.

Table I

Endocrine activity of prothoracic glands removed at the outset of adult development and tested in brainless pupae or in pupal abdomens

Test preparation	Pairs of glands implanted into each	Number of experiments	Number developing
Pupal Cecropia abdomens	3 from Cecropia	2	1
	3 from Polyphemus	4	2
Brainless diapausing Cecropia pupae	2 from Cecropia	1	0
	2 from Polyphemus	2	0
	3 from Cecropia	2	1
	3 from Polyphemus	3	3
		14	7

Prothoracic glands were therefore removed from animals immediately after the onset of adult development and implanted into isolated pupal abdomens. Experiments of this type were complicated by the fragility and stickiness of the glands which, at the outset of adult development, begin to undergo rapid degeneration;

[2] Prepared daily by the addition of 1 part 0.1 *M* potassium cyanide solution to 9 parts of the insect Ringer's solution described by Ephrussi and Beadle (1936). At the pH of the insect, the cyanide exists almost wholly as HCN and within a few days is lost *via* the tracheal system.

ordinarily they could be isolated and implanted only as small fragments. However, by using *Telea polyphemus* as donors, the difficulty was somewhat lessened, since, in this closely related diapausing species, the prothoracic glands are much easier to locate and isolate during the first few days af adult development.

A further serious complication was the high mortality among abdomens receiving such implants. Notwithstanding the use of cyanide and phenylthiourea, the implanted glands caused a delayed darkening of the blood followed by the death

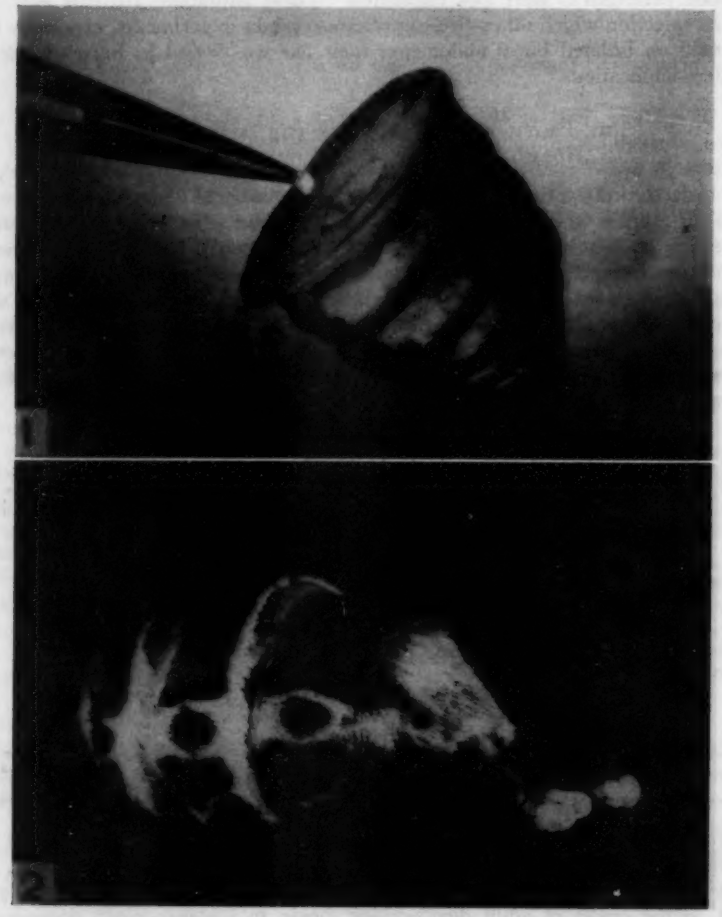


FIGURE 1. Isolated abdomen of a diapausing Cecropia pupa sealed to a plastic slip. Prothoracic glands obtained from postdiapausing pupae are being implanted through a central hole in the slip.

FIGURE 2. The preparation in Figure 1, after adult development. The active prothoracic glands have evoked the metamorphosis of the abdomen; the latter is shown laying several eggs.

of the preparation. This result was not peculiar to the prothoracic glands, but was also commonly observed after implanting fragments of fat-body obtained from developing adults.

Of a total of 25 isolated pupal abdomens, only 6 survived longer than a week following the implantation of prothoracic glands obtained from developing animals (Fig. 1). However, as may be observed in Table I, three of these underwent prompt and complete adult development (Fig. 2).

A lower mortality was encountered in experiments testing the glands in brainless diapausing pupae rather than in isolated abdomens. Of a total of 8 viable preparations of this type, four underwent adult development (Table I).

In view of the injury and fragmentation of the glands during the experimental procedure, the high proportion of negative results is understandable. However, the fact that adult development occurred in 7 of the total of 14 viable preparations is, in itself, highly significant. It demonstrates that the pupal tissues do not require prior reaction with the brain hormone in order to respond to the prothoracic gland hormone.

Ligation Experiments on Mature Larvae

Efforts were made to determine whether the brain and prothoracic glands control pupation as well as adult development. In view of the difficulties inherent in surgical procedures on caterpillars, the technique of ligation was applied to mature fifth instar Cecropia silkworms.

As illustrated in Figure 3, two transverse ligatures were placed around each larva, one between the head and prothorax and the other between the second and third abdominal segments. In this manner each animal was subdivided into three blood-tight compartments—a cephalic compartment containing the brain, corpora cardiaca, and corpora allata; a primarily thoracic compartment containing the prothoracic glands; and an abdominal compartment containing no recognized endocrine glands.

The isolated head promptly died, but, as indicated in Table II, the behavior of the thoracic and abdominal compartments was determined by the stage of maturity of the silkworm at the time of ligation. Larvae subdivided just prior to the initiation of spinning underwent no further development, although both thoracic and abdominal compartments continued to live for about a month thereafter (Fig. 3). When the ligatures were applied during the first day of spinning, the abdomen remained larval, whereas the thorax either remained larval or pupated during the following two weeks. Silkworms subdivided during the second day of spinning retained larval abdomens, but all of the thoracic compartments underwent pupation about two weeks thereafter (Fig. 4).

The pupation of the larval abdomen became possible only when the ligatures were applied after the initiation of the prepupal stage. At 25° C. this stage begins four to five days after the initiation of spinning and is accompanied by the eversion of the wing disks and the retraction of the hypodermis from the larval cuticle. In the intact animal this retraction may be recognized by the withdrawal of the pigment granules underlying the transparent larval ocelli (Kühn and Piepho, 1936). Silkworms ligated at the first sign of pigment retraction retained larval abdomens

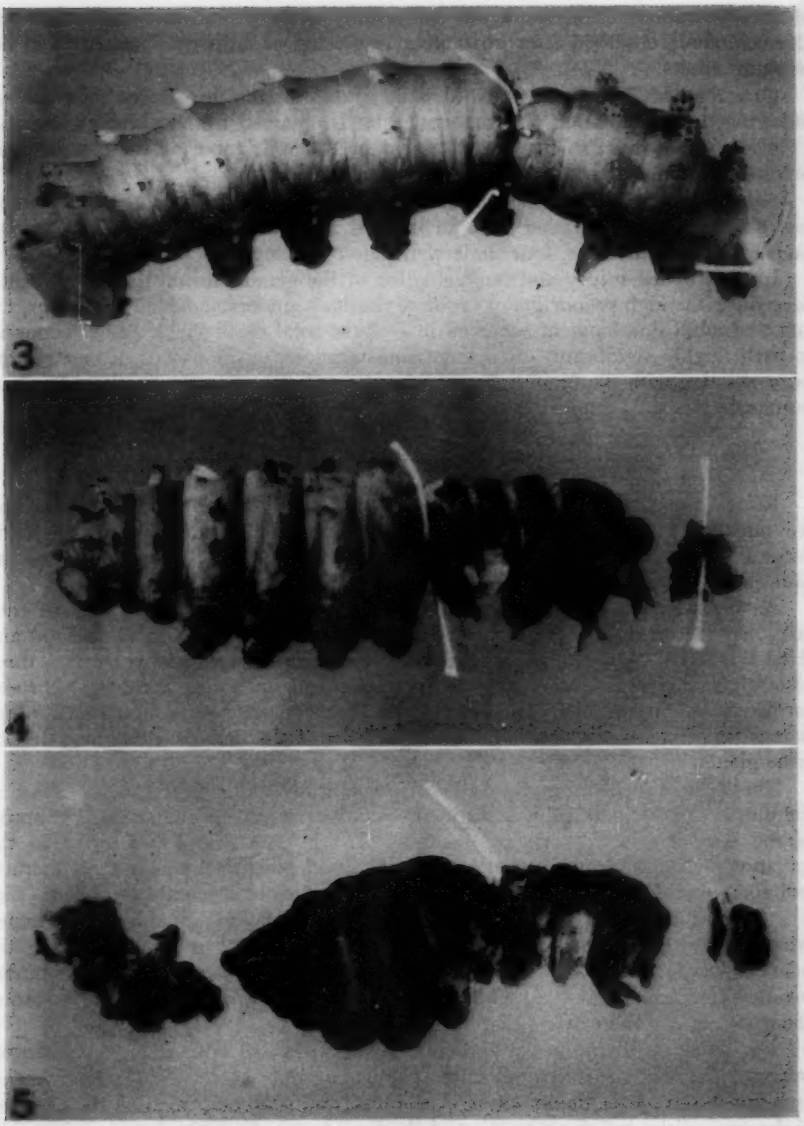


FIGURE 3. A mature Cecropia larva ligatured behind head and thorax prior to the initiation of spinning. Neither end is able to metamorphose.

FIGURE 4. The same as Figure 3, except that the ligatures were applied during the second day of spinning. The thorax has pupated, but the abdomen remains larval.

FIGURE 5. The same as Figure 3, except that the ligatures were applied after the initiation of the prepupal stage. Both the thorax and abdomen have pupated. The dead larval head and moulted larval cuticle are also illustrated.

in 17 of the 21 preparations. However, in animals ligated a day later, pupation of the abdominal compartment was consistently observed (Fig. 5).

These simple experiments demonstrate that pupation is under the control of both the head and the thorax. The head makes its contribution prior to a critical period signalled by the completion of the outer capsule of the cocoon and, thus, 9 days before the pupal moult. Pupation then comes under the control of the thorax until a critical period signalled by the onset of the prepupal stage.

TABLE II

Behavior of thoracic and abdominal compartments of double-ligatured Cecropia silkworms

Days at 25° C.	Corresponding stage	Number of experiments	Final state of	
			Thorax	Abdomen
0	Just prior to spinning	5	Larval	Larval
0 to 1*	Spinning outer capsule	14	Larval or pupal	Larval
1 to 2	Spinning inner capsule	18	Pupal	Larval
2	Just finished spinning	17	Pupal	Larval
4 to 5**	Earliest retraction of ocellar pigment	21	Pupal	Larval or pupal
5	Full retraction of ocellar pigment	4	Pupal	Pupal
5 to 9	Prepupa	4	Pupal	Pupal
9 to 10	Pupal moult	—	Pupal	Pupal

* "Critical period" for brain.
** "Critical period" for prothoracic glands.

Those thoracic or abdominal compartments which pupated underwent no further development though they continued to live for up to three months thereafter. This behavior is understandable since, as we have seen, the pupal-adult transformation once again requires the hormonal participation of a cephalic organ, the brain, and a thoracic organ, the prothoracic glands.

Ability of Larval Brains to Terminate Pupal Diapause

Efforts were made to determine whether the larval brain controls pupation *via* the same brain hormone, which, at a later stage in the life history, controls adult development. To this end, brains were dissected from caterpillars at precise stages, freed from frontal ganglia and other attached organs, and implanted into the tips of the abdomens of brainless diapausing pupae. Two brains were implanted into each preparation.

The results recorded in Table III demonstrate the ability of the larval brain to substitute for the pupal brain in terminating diapause. In one or more experiments, the brains were found active at every stage in larval and prepupal life.

However, it will also be observed in Table III that many of the brain implants failed to promote adult development and, even when they did so, considerable variation was encountered in the time required for the initiation of development.

When observed through an overlying plastic window, it became apparent that the implanted brains attained intimate connections with the tissues of the host prior to exerting their effect. Minute nerve fibers grew out from the brain and tracheal connections grew in from the surrounding tissues of the host. The subsequent history of such implants was therefore largely uncontrollable—a fact which was probably responsible for a large fraction of the observed variability.

TABLE III

Endocrine activity of larval brains implanted into brainless diapausing Cecropia pupae (two brains into each)

Stage of donors	Number of experiments	Number developing	Days for initiation of development* at 25° C.
Mature 1st instar	2	1	50
1st moulting to 2d	3	1	195
Early 2d	2	2	20; 251
Mature 2d	3	1	17
2d moulting to 3d	2	2	13; 226
Early 3d	1	1	228
3d	2	2	10; 181
Mature 3d	3	2	21; 139
3d moulting to 4th	2	2	25; 217
Early 4th	2	2	28; 110
4th	2	2	48; 257
Mature 4th	4	3	16; 51; 173
4th moulting to 5th	4	3	65; 177
Early 5th	17	12	12; 18; 21; 23; 25; 47; 48; 58; 73; 112; 225;—
Mature 5th	18	9	11; 48; 107; 137; 143; 180; 218; 221; —
Spinning outer capsule	19	8	12; 17; 18; 28; 39; 55; 112; 190
Spinning inner capsule	13	6	65; 71; 200; 210; 240; 520
Finished spinning	11	5	65; 129; 134; 152; 166
Early retraction of ocellar pigment	6	5	80; 97; 130; 153; 187
Prepupa	7	5	64; 140; 159; 165; 225
Fresh pupa	10	0	—

* Positive experiments only.

Consequently, on the basis of the present data, it is impossible to judge whether any systematic change occurs in the brain's endocrine activity between the first larval stage and the initiation of spinning. However, it is clear that during the first day of spinning the brain is as active as at any stage in larval life. Yet, within the nine days that follow, a rapid decline takes place in its endocrine activity. By the time of the pupal moult, the brain is totally inactive when tested. The net result is that the newly formed pupa is equipped with a brain which is incompetent to secrete the brain hormone.

Ability of Larval Prothoracic Glands to Terminate Pupal Diapause

According to the preceding analysis, the larval brain secretes the same brain hormone in promoting pupation as does the pupal brain in promoting adult de-

velopment. An extension of this principle to the prothoracic glands suggests that the same prothoracic gland hormone controls both pupation and adult development. This hypothesis was tested in brainless diapausing pupae by implanting prothoracic glands obtained from caterpillars at various stages during late larval and prepupal life. Two pairs of glands were implanted into each pupa.

As recorded in Table IV, larval glands removed prior to the initiation of spinning caused adult development to occur in only two of the 23 preparations, whereas glands removed after the initiation of spinning and prior to the prepupal period were active in 7 of 11 preparations. Glands removed after the onset of the prepupal stage were inactive.

TABLE IV

Endocrine activity of larval prothoracic glands implanted into brainless diapausing cecropia pupae (two pairs into each)

Stage of donors	Number of experiments	Number developing	Days for initiation of development* at 25° C.
Mature 4th instar	4	0	—
4th moulting to 5th	2	0	—
Early 5th	9	1	27
Mature 5th	8	1	19
Spinning outer capsule	2	2	16; 21
Spinning inner capsule	5	2	15; 16
Finished spinning	3	3	23; 240; 480
Early retraction of ocellar pigment	3	0	—
Prepupa	1	0	—
Pupa	5	0	—

* Positive experiments only.

From these experiments we learn that the larval prothoracic glands can be made to substitute for the pupal glands in terminating pupal diapause. Since the glands were tested in brainless pupae, the experiments also demonstrate that the larval glands usually exist in the activated state during a five-day period beginning with the initiation of spinning and ending with the onset of the prepupal stage.

PUPATION INDUCED BY PUPAL ENDOCRINE ORGANS

In the experiments just considered, the larval brain and prothoracic glands were able to provide the necessary hormonal stimulus for the adult development of the pupa. Can the corresponding pupal organs evoke the pupation of the larva?

A preliminary series of experiments indicates an affirmative answer to this question. In three of a total of six preparations, "permanent" larval abdomens, isolated by ligation prior to spinning, were induced to pupate by implanting brains and prothoracic glands obtained from chilled pupae.

Parabiosis Experiments

In our progress up to this point, support has been found for the view that the prothoracic glands secrete the metamorphosis hormone under tropic stimulation of the brain hormone. Further insight into the function of this endocrine system is afforded by experiments where brainless pupae were joined in serial parabiosis.

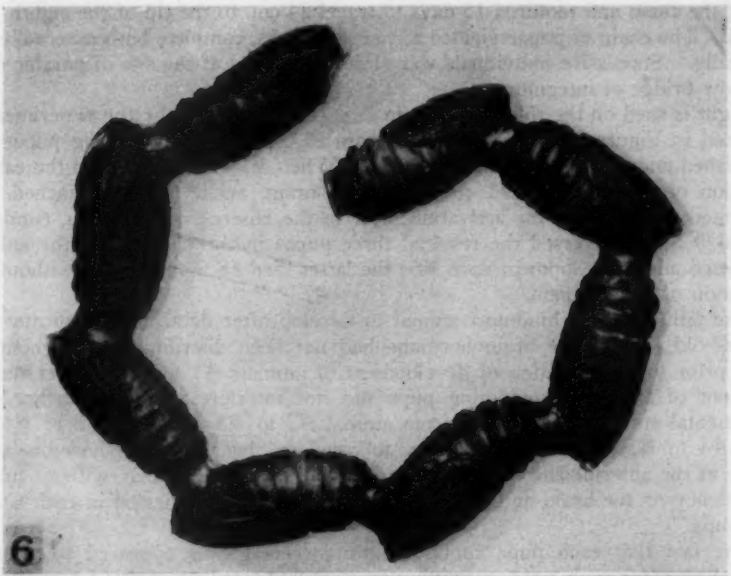
A series of Cecropia pupae were first stabilized in "permanent" diapause by removing their brains and replacing the pupal cuticle and hypodermis at the site of the operation by a transparent plastic facial window (Williams, 1946b, 1947). In like manner, plastic windows were established in each pupa at the thoracic tergum and the tip of the abdomen. A week later, a window was removed from each individual and the pupae united in pairs, the thorax of the one being sealed with melted paraffin to the tip of the abdomen of the other. Ten days later the pairs of pupae were united by a continuation of the above-mentioned procedure. In this manner, chains of four to ten brainless pupae were established in serial parabiosis.[3] In approximately a week the operative sites underwent a process of repair accompanied by a growing together of the hypodermis of successive individuals, yielding, as it were, a single elongate organism possessing continuity of blood and hypodermis. Though each individual retained prothoracic glands within its thorax, none possessed a brain within its head. For this reason the diapause was persistent and three chains of pupae died several months later without any indication of adult development.

Experiments were performed to ascertain the effects of implanting a brain into such preparations. Accordingly, a single brain from a previously chilled pupa was implanted under the facial window of the first animal in each chain, its effects being noted by daily observations through the transparent facial windows. The five viable preparations of this type yielded essentially similar results. The behavior of the preparation illustrated in Figures 6 and 7 may be summarized as follows:

0 day—brain implanted into #1.
17th day—#1 and #2 show initiation of development; no development of #3 to #8.
20th day—#3 and #4 show initiation of development; no development of #5 to #8.
21st day—#5 shows initiation of development; no development of #6 to #8.
30th day—#6 and #7 show initiation of development; no development of #8.
32nd day—#8 shows initiation of development.
38th day—#1 and #2 have completed adult development.
41st day—#3, #4, and #5 have completed adult development.
50th day—#6 and #7 have completed adult development.
52nd day—#8 has completed adult development.

Thus we observe that the single brain set in motion a process of activation which terminated the diapause of each animal in turn. This process began at the anterior

[3] The principal difficulty in establishing these chains is that an infection or darkening of the blood in any one animal promptly spreads throughout the chain and causes the death of all individuals. It is therefore advisable to subdivide the total procedure into several stages spaced at least a week apart. In this manner one may confirm the viability of each animal before proceeding to the next step in assembling the chain.



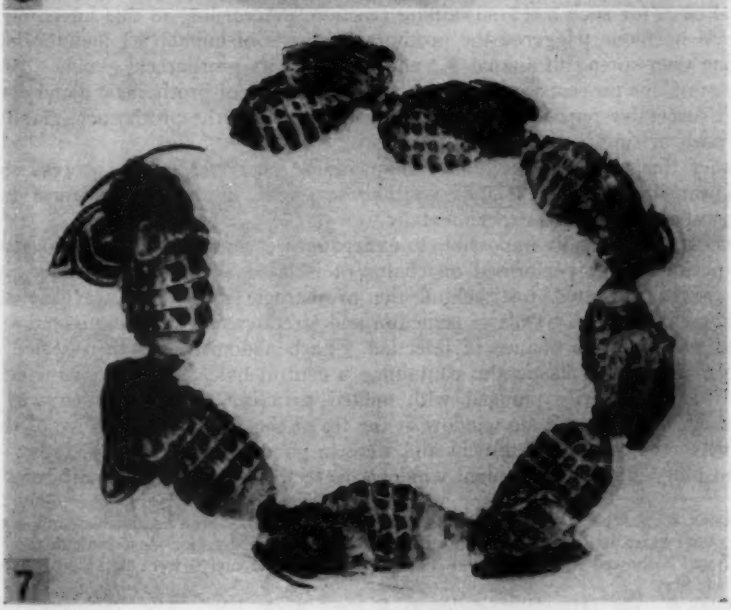


FIGURE 6. A chain of eight brainless diapausing pupae has been established and a single chilled pupal brain implanted under the facial window of the anterior-most individual. Approximately life size.

FIGURE 7. The preparation in Figure 6 after seven and one-half weeks. The single brain has caused all the pupae to undergo adult development.

end of the chain and required 15 days to travel 24 cm. to the tip of the eighth individual. The chain of pupae yielded a chain of moths, complete both externally and internally. Successive individuals were inter-connected at the site of parabiosis by a hollow bridge of integument.

Light is shed on the role of the brain in such preparations by the experiment illustrated in Figures 8 and 9. Here a chain of 6 brainless diapausing pupae was established and a brain implanted into #1. When #1 and #2 showed the earliest initiation of development, #1 containing the brain, and #6 were detached. As illustrated in Figure 9, the activation, now in the absence of the brain, continued beyond #2 and traversed the residual three pupae in the chain. All the animals completed adult development save #6; the latter died 18 months later without any indication of development.

The failure of the hindmost animal to develop after detachment indicates that a threshold titer of the brain hormone had not been distributed throughout the chain prior to the initiation of development of animals #1 and #2. Yet the detachment of the brain-containing pupa did not interfere with the further non-decremental spread of activation from animal #2 to #3 and from #3 to #4 and from #4 to #5. It seems necessary to conclude that the brain hormone acted locally at the anterior end of the chain to initiate a reaction which, without further dependency on the brain or the brain hormone, could be duplicated in each successive pupa.

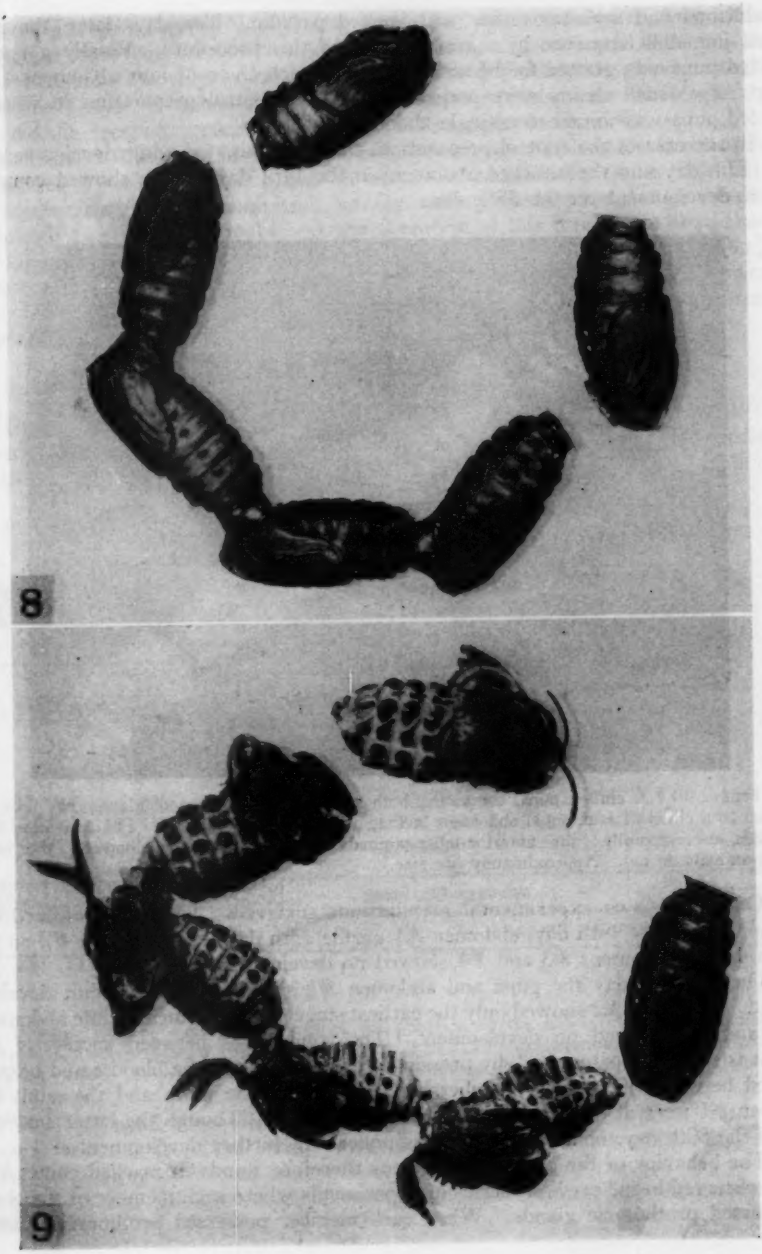
The fact that each pupa contained prothoracic glands appeared to afford a rational basis for such a self-sustaining reaction. According to this interpretation, the brain hormone triggered the prothoracic glands of animal #1 and the latter's hormone then spread to animal #2 and triggered its prothoracic glands. A continuation of this process would lead to the recruitment of prothoracic gland activity in each successive pupa—the glands being triggered by the prothoracic gland hormone itself arising in the preceding pupa.

Under this point of view, the self-sustaining character of the process should not appear if prothoracic glands were present only in the anterior-most insect. This prospect was tested experimentally.

Since it is technically impossible to extirpate the prothoracic glands of Cecropia, the experiment was performed on chains of isolated abdomens. Twenty chilled pupae were transected just behind the prothoracic glands; *i.e.*, at the meso-metathoracic level. Crystals of penicillin and streptomycin were implanted at this time to minimize the chance of infection. Each abdomen was then sealed with melted paraffin to a plastic slip containing a central hole 5 mm. in diameter; the latter was temporarily plugged with melted paraffin. Each abdomen was further provided with a plastic window at the tip of the abdomen. Twelve of the 20 abdomens survived this treatment and were in good condition a week later. The paraffin plugs and the abdominal windows were then removed and pairs of pupae

FIGURE 8. A chain of six brainless diapausing pupae has been established and a single chilled pupal brain implanted under the facial window of the anterior-most individual. When pupae 1 and 2 showed the initiation of development, pupae 1 and 6 were detached. Approximately life size.

FIGURE 9. The preparation in Figure 8 seven weeks later. After detachment of the brain-containing pupa, the activation has continued to spread down the chain of interconnected pupae. The hindmost pupa fails to develop after detachment. Approximately life size.



established and sealed together with melted paraffin. Ten days later, the pairs were joined in sequence by a continuation of this procedure. Finally a normal chilled pupa was grafted to the anterior end of each chain of four abdomens (Fig. 10). Two such chains were prepared, plus one control preparation in which a chilled pupa was joined to a single abdomen.

In the case of the control preparation, the pupa initiated adult development on the 12th day and the attached abdomen on the 14th day. Both showed complete adult development on the 35th day.

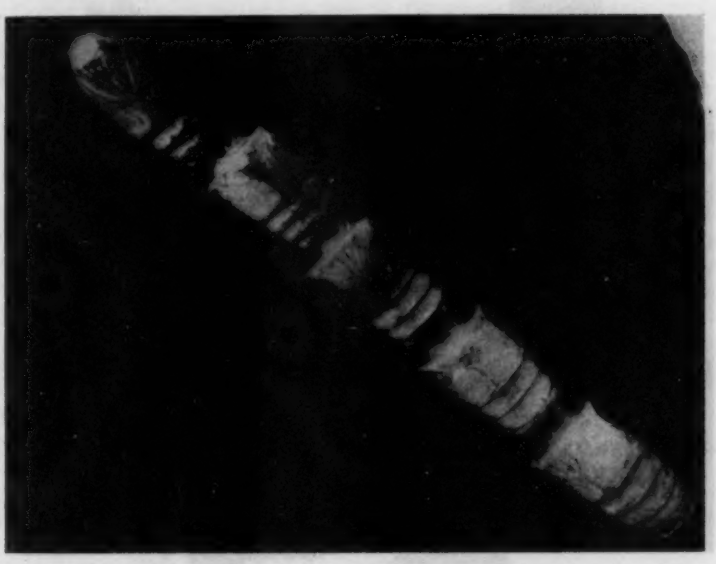


FIGURE 10. A chilled pupa, containing both a brain and prothoracic glands, has been attached to a chain of four pupal abdomens lacking these endocrine organs. The activation here spreads decrementally: the anterior-most animals undergo adult development, the hindmost animals do not. Approximately life size.

One of the two experimental preparations survived. The pupa initiated development on the 14th day, abdomen #1 on the 15th day, and abdomen #2 on the 18th day. Abdomens #3 and #4 showed no development.

On the 38th day the pupa and abdomen #1 showed complete adult development. Abdomen #2 showed only the earliest stage of development, while abdomens #3 and #4 showed no development. The connections between successive abdomens remained patent and, by pressing on the abdomens, the blood could be propelled between the various members of the chain. The moth and the adult abdomen #1 were at this time detached from the chain. Though the latter survived until the 80th day, none of its members underwent further development.

The behavior of the chain of abdomens therefore stands in marked contrast to that observed in the previous series of experiments where each member of the chain possessed prothoracic glands. When each member possessed prothoracic glands,

then the activation spread without decrement and could apparently cause the development of any number of brainless pupae that one incorporated into the chain. But when only the anterior-most animal possessed prothoracic glands, then the activation spread decrementally and only the next adjacent member of the chain received the necessary concentration of prothoracic gland hormone.

Just such a difference in the behavior of the two types of preparations would be anticipated if the prothoracic glands of successive individuals can be triggered by the prothoracic gland hormone itself, arising in the preceding member of the chain. Manifestly, within the normal insect the operation of this mechanism would assist the integration of the endocrine mechanism. Since the prothoracic glands are paired organs, whose respective thresholds to brain hormone may differ, the sensitivity of one prothoracic gland to the other's hormone would serve to synchronize their secretory activities and couple the two glands into a functional unity.

Discussion

The experimental results summarized in Figure 11 demonstrate that both pupation and adult development are controlled by an endocrine system consisting of the brain and prothoracic glands. According to the ligation experiments

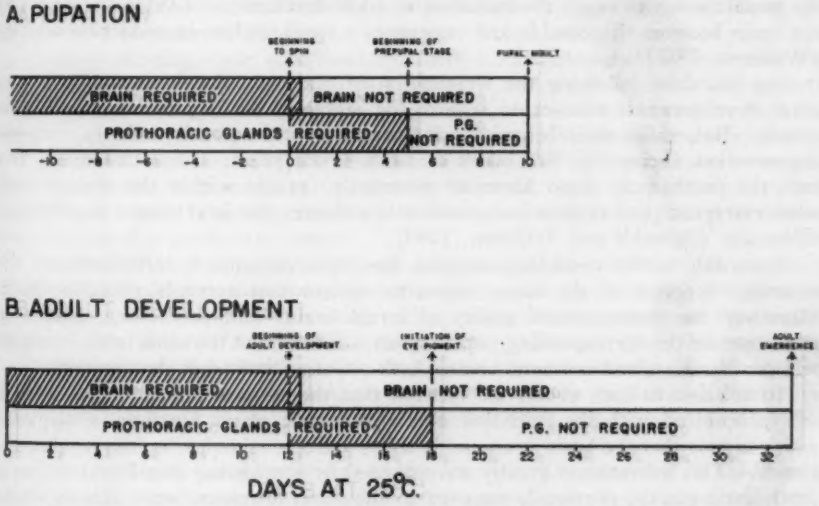

A. PUPATION
BEGINNING TO SPIN
BEGINNING OF PREPUPAL STAGE
PUPAL MOULT
BRAIN REQUIRED
BRAIN NOT REQUIRED
PROTHORACIC GLANDS REQUIRED
P.G. NOT REQUIRED
-10 -8 -6 -4 -2 0 2 4 6 8 10
B. ADULT DEVELOPMENT
BEGINNING OF ADULT DEVELOPMENT
INITIATION OF EYE PIGMENT
ADULT EMERGENCE
BRAIN REQUIRED
BRAIN NOT REQUIRED
PROTHORACIC GLANDS REQUIRED
P.G. NOT REQUIRED
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
DAYS AT 25°C.

Figure 11. The endocrine control of the pupation of the larva (A) and the adult development of the previously chilled pupa (B) at 25° C. For explanation see text. The cross-hatching records the periods when the brain or prothoracic glands were found maximally active.

(Table II), pupation requires an initial stimulus from the larval brain and a subsequent stimulus from the larval prothoracic glands. As diagrammed in Figure 11A, the brain completes its contribution to pupation during the first day of spinning, *i.e.*, 9 days prior to the pupal moult, and then declines in endocrine activity (Table III).

As also illustrated in Figure 11A, the termination of brain function ushers in a five-day period of prothoracic gland function, beginning with the onset of spinning and ending with the onset of the prepupal stage. The further development of the pupa then becomes independent of the prothoracic glands. The latter remain intact, but show a rapid loss of endocrine activity (Table IV).

Within the pupa the function of the prothoracic glands in promoting adult development once again requires the tropic stimulus of the brain hormone. But in the Cecropia silkworm, as we have seen, this stimulus is not forthcoming for several months after pupation, until the diapausing brain has recovered its endocrine competency under the influence of low environmental temperature.

When such a chilled pupa is placed at 25° C., the events which terminate its diapause are strikingly similar to those which controlled its pupation. As diagrammed in Figure 11B, the secretion of the brain hormone occurs promptly at the high temperature, proceeding at a rate determined largely by the duration of the brain's prior exposure to low temperature (Williams, 1952). The brain hormone attains threshold titer after a specific period, diagrammed as 12 days in Figure 11B. Then, within a few hours, a remarkable series of events takes place: (1) the prothoracic glands, which have been inactive since the prepupal stage, are triggered by the brain hormone; (2) the prothoracic gland hormone reacts with the pupal tissues to evoke the initiation of adult development (Table I); and (3) the brain becomes dispensable and undergoes a rapid decline in endocrine activity (Williams, 1947).

For six days following the termination of diapause, the further progress of adult development continues to require the secretory activity of the prothoracic glands. But, unlike their behavior prior to pupation, the glands undergo complete degeneration during this first week of adult development. Notwithstanding this fact, the prothoracic gland hormone apparently persists within the animal until adult emergence and is thus in a position to influence the later stages of adult development (Schmidt and Williams, 1949).

According to the preceding analysis, the pupal diapause is terminated by the recurrent function of the same endocrine system that controls pupation itself. Moreover, the demonstrated ability of larval brains and prothoracic glands to substitute for the corresponding pupal organs indicates that the same brain hormone and prothoracic gland hormone control both pupation and adult development.

In relation to both events we observe that the termination of brain function is synchronized with the initiation of a prothoracic gland function—a sequence which argues that the brain hormone is solely a tropic stimulus for the prothoracic glands. This inference is greatly strengthened by the finding that larval or pupal prothoracic glands, previously exposed to the brain hormone, were able to evoke adult development in the absence of the brain (Table I). Though the brain gives leadership to the endocrine events which preside over pupation and adult development, it apparently does so *via* the tropic action of its hormone on the prothoracic glands.

In agreement with Fukuda's investigation of *Bombyx mori* (1944), the present findings therefore serve to emphasize the significance of the prothoracic gland hormone, since it is apparently this factor which reacts with the larval tissues to induce pupation and with the pupal tissues to induce adult development.

Attempts to determine whether the brain and prothoracic glands also participate in the hormonal control of larval growth and moulting are complicated by technical difficulties. For as Bounhiol (1938) has emphasized, the moulting or pupation of most insects becomes possible only when the animal attains a certain nutritional status. Each larval instar therefore begins with a period of *alimentation indispensable,* followed by a period of *alimentation facultative.* During the first of these periods metamorphosis is blocked by any treatment which prevents further feeding—such measures, for example, as ligation, brain removal, or simple starvation. In consequence, a direct approach to the analysis of the hormonal control of moulting has proved feasible only in *Rhodnius* where, at the beginning of each instar, the period of *alimentation indispensable* is limited to a single blood meal (Wigglesworth, 1940, 1951). Studies of this type have proved difficult or impossible in most other insects where the hormonal control of moulting is usually exercised during the period of *alimentation indispensable.*

Indeed, our understanding of the hormonal control of pupation is obscured by this difficulty in certain species. Unfortunately, this appears to be so in a favorite experimental animal, *Bombyx mori.* Here the brain apparently secretes a threshold titer of its hormone early in the final larval instar when most experimental procedures are impossible. This fact was adequately appreciated by Bounhiol (1938) and probably accounts for Fukuda's (1944) failure to demonstrate a role of the brain in the pupation of *B. mori.*

Notwithstanding these complications, there is circumstantial evidence that the brain and prothoracic glands also participate in the regulation of larval growth and moulting when, in the immature insect, they function in conjunction with the corpora allata's "juvenile" or "status quo" hormone. This conclusion is supported by two lines of evidence: (1) the demonstrated ability of Cecropia brains to secrete the brain hormone as early as the first larval instar; and (2) Fukuda's studies (1944) of *B. mori* where the moulting of the immature insect appeared to require the function of a prothoracic center.

Thus, at all stages in post-embryonic development, the picture which gradually takes shape finds the prothoracic glands supplying an apparently direct stimulus for cellular growth and differentiation. Functioning in the presence of the corpora allata's "juvenile" or "status quo" hormone, the prothoracic gland hormone promotes the growth and moulting of the immature larva; functioning in the absence of this conservative factor, it promotes the rapid strides in growth and differentiation which culminate in the pupation of the mature larva and the adult development of the pupa.

This conclusion based on studies of the Cecropia silkworm and its relatives is in substantial agreement with Fukuda's (1944) results on *B. mori* and will probably apply to all Lepidoptera. Indeed, recent studies of the Diptera (Possompes, 1949, 1950a, 1950b), the Blattoidea (Bodenstein, 1951), and Hemiptera (Wigglesworth, 1951) suggest that, in all metamorphosing insects, the prothoracic glands and their homologues are the source of the factor which has variously been termed the "moulting," "pupation," or "growth and differentiation" hormone.

Consequently, the control which the prothoracic gland hormone exercises over pupation and adult development in the Cecropia silkworm is, most probably, the expression of a generalized phenomenon. The pupal diapause, under this point of view, finds an endocrine basis in the latent operation of a normal mechanism shared

with non-diapausing species—the secretion of the prothoracic gland hormone. And according to the results of the present investigation, this latency, in turn, results from a loss of the endocrine activity of both the brain and the prothoracic glands during the prepupal period. Adult development becomes possible only when the brain recovers its secretory powers and repeats the tropic stimulation which it had administered to the prothoracic glands prior to the prepupal period.

In the case of non-diapausing species, where the prothoracic glands promote prompt adult development by secreting their hormone a few days following pupation, the timing of the endocrine events appears to be modified in one of three directions: (1) both the brain and prothoracic glands may remain active following pupation; or (2) the brain may remain active within the newly found pupa while the prothoracic glands become temporarily inactive; or (3) the brain may become inactive following pupation while the prothoracic glands remain active. All of these circumstances are consistent with the function or activation of the prothoracic glands and, therefore, with the prompt onset of adult development; all will probably be encountered when a sufficiently large number of non-diapausing species is examined.

In the case of species incapable of pupal diapause, the first or third adaptations appear to be the rule. Thus the adult development of *Bombyx* (Bounhiol, 1938; Fukuda, 1944), *Deilephila* (Caspari and Plagge, 1935; Plagge, 1938), *Galleria* (Bounhiol, 1938), *Lymantria* (Kopeć, 1922; Bounhiol, 1938), *Phryganidia* (Bodenstein, 1938), and *Vanessa* (Hachlow, 1931) is known to proceed normally in brainless pupae. Our studies show this to be true also in the non-diapausing pupae of the monarch butterfly, *Danaus plexippus*, and the southern armyworm, *Prodenia eridania*.

In the case of the bivoltine silkworm, *Actias luna*, and the polyvoltine *Actias selene*, the initial brood of non-diapausing pupae undergoes prompt adult development *via* the second adaptation mentioned above. Within the newly formed pupa the brain is active and the prothoracic glands inactive. The pupa is therefore converted to diapause if the brain is removed immediately after pupation. However, the subsequent diapausing brood of *Actias luna* shows the same timing of events as observed in Cecropia in that the brain as well as the prothoracic glands are inactive within the newly formed pupa.

Thus, in our understanding of the endocrinology of metamorphosis, we simultaneously perceive a simplification and an increasing complexity—simplification in the operation of a potent growth factor, the prothoracic gland hormone, in controlling growth and differentiation at all stages in post-embryonic development; increasing complexity in that secretion of this hormone is under the tropic control of the brain.

The photographs in Plate I, II, and V were made in collaboration with Dr. Roman Vishniac and are used with the permission of *Time*, Inc. Mr. Dietrich Bodenstein, Dr. Leigh E. Chadwick, and Dr. Berta Scharrer were most helpful in reading the paper in manuscript form; the presentation has profited greatly by their criticisms and suggestions.

Summary

1. The pupation and adult development of the Cecropia silkworm are under the control of a hormonal system consisting of the brain and prothoracic glands. The adult development of the pupa is controlled by the same "brain hormone" and "prothoracic gland hormone" which, at an earlier stage in the life history, control the pupation of the larva.

2. Though the brain gives leadership to the endocrine events that preside over pupation and adult development, it apparently does so by supplying a tropic hormonal stimulus for the prothoracic glands. The latter's "growth and differentiation hormone," secreted under stimulation of the brain hormone, then reacts with the larval tissues to promote pupation or with the pupal tissues to promote adult development.

3. The pupal diapause is a state of endocrine deficiency resulting from a temporary failure of the brain in secreting its tropic hormone following pupation. What the pupal tissues require is the prothoracic glands' growth and differentiation hormone. But this factor becomes available only after the brain recovers its secretory ability and triggers the function of the prothoracic glands.

4. Evidence is presented that the prothoracic glands can be triggered also by the prothoracic gland hormone itself. This device, it is suggested, serves to couple the paired glands into a functional unity and assure their simultaneous response when the brain hormone is released.

5. Evidence of a more circumstantial character indicates that larval growth and moulting are also promoted by the prothoracic glands' growth and differentiation hormone, acting within the immature insect in conjunction with a conservative factor secreted by the corpora allata. The corpus allatum hormone plays no positive role in the pupation or adult development of the Cecropia silkworm.

6. The endocrine system of Cecropia is compared to that of bivoltine and non-diapausing Lepidoptera. In Cecropia, both the brain and prothoracic glands become inactive after providing the endocrine stimulus for pupation. Prompt, non-diapausing development becomes possible in those species where either the brain or the prothoracic glands retain their endocrine activity within the newly formed pupa.

7. The pupal diapause therefore results from the delayed function of a normal endocrine mechanism shared with non-diapausing species.

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THE PROTOPLASMIC CORTEX IN RELATION TO STIMULATION [1]

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In spite of the fact that the protoplasmic cortex is undoubtedly a most important part of the cell, little is known concerning its physical properties and how these may change during the life and activity of the cell. Typically, living cells consist of a mass of fluid protoplasm surrounded by a stiff layer of protoplasm just underneath the cell membrane. This stiff layer constitutes the cortex. A recognizable cortex has been described for ameba and for various types of marine egg cells. Such a cortex also exists in striated muscle cells (Rieser, 1949), and there is a likelihood that all cells have a cortex. In a marine egg cell the organization of the embryo and the course of development may well depend on the physical arrangement of the cortex, for this is often the only part of the egg protoplasm that is firm, and it is difficult to conceive of organization in a highly fluid mass of material. In irritable cells, the cortex may be the sensitive portion of the living structure. This would be a logical enough assumption, for the outer portion of the protoplasm presumably would be the first to become affected by external stimuli.

The colloidal theory of stimulation and response states that typically the first action of a stimulating agent is on the cortex of the cell. The stimulus causes a release of bound calcium from the cortex and this calcium passing into the cell interior causes changes in the protoplasm there. The evidence for this theory is many-sided and need not be discussed here (see Heilbrunn, 1951, for a recent summary). In the ameba, it is certain that various types of stimulating agents do cause a liquefaction of the cortex, and this liquefaction is thought to be associated with a release of calcium ion. But a weakness of the colloidal theory lies in the fact that it is only in the ameba that such changes in the cortex have actually been observed. This is due to the fact that although various types of cells are known to have a cortex, this cortex is ordinarily so stiff that it can not readily be studied.

Fortunately the egg of the worm *Chaetopterus pergamentaceus* has a cortex with easily visible granules and these can be moved by centrifugal forces not violent enough to break the cell. By measuring the amount of centrifugal force necessary to move granules out of the cortex, it is possible to estimate the stiffness of the cortical gel under one condition or another (compare Heilbrunn, Wilson and Harding, 1951, and especially Wilson, 1951).

We were interested in finding out first whether the cortex of the Chaetopterus egg cell was similar in its properties to the cortex of the ameba. The ameba cortex is liquefied by agents which tend to remove calcium from it (oxalates or potassium salts); it becomes stiffer in the presence of excess calcium. Likewise both heat and

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cold cause a liquefaction of the ameba cortex; ultraviolet radiation has a similar effect, and so also do fat solvents. We wanted to know therefore whether or not the Chaetopterus egg cortex behaves in this manner.

Secondly in view of our belief that the response to stimulation which occurs when a cell is induced to divide as a result of treatment with various agents is similar to the response to stimulation which occurs generally in various types of irritable protoplasm, we thought that a proper study of the Chaetopterus egg cortex might help us to understand why it is that so many different types of agents can cause an egg cell to undergo division. It is true that in earlier publications an attempt has been made to interpret the initiation of cell division in terms of the colloid chemical theory of stimulation (see Heilbrunn, 1937, 1943, 1952). But when this earlier discussion was written, there was no direct evidence that an egg cell when it was exposed to a stimulating agent behaved like an ameba. The present study is an attempt to study that evidence.

We can say at the outset that our study has been successful from both standpoints. As we shall proceed to show, the cortex of the Chaetopterus egg does behave very much like the cortex of the ameba. And secondly we have found ample support for the view that initiation of cell division can be interpreted in terms of the colloidal theory of stimulation. For the most varied types of stimulating agents do cause both an initiation of division and a liquefaction of the cortex.

As in our earlier work (Heilbrunn, Wilson and Harding, 1951; Wilson, 1951) we estimated the stiffness of the cortex by determining the force necessary to move granules out of it. With the aid of an Emerson hand centrifuge,[2] eggs were exposed to varying centrifugal forces for a minute. Then they were examined under high power of the microscope. Generally speaking, each of us examined ten centrifuged eggs, and from the twenty eggs observed we calculated the percentage of eggs which showed a definite movement of granules from the cortex. We made one series of tests for the eggs in the solutions whose effects we were testing and another series of tests for the control eggs in sea water. The end point was taken as that centrifugal force just sufficient to cause movement of granules from the cortex. In order to get this exact value, we usually resorted to interpolation. For example, if we found that turning the centrifuge 40 times in a minute caused 40% of the eggs to show movement of granules from the cortex, and turning the centrifuge handle 42 times in a minute caused 60% of the eggs to show movement of cortical granules, then the end point was taken as the force developed by turning the centrifuge handle 41 times per minute. No claim is made for the exactness of our values, but in every case repetition of the experiments gave us concordant results. One difficulty in performing the experiments arose from the fact that in the control eggs the rigidity of the cortex kept decreasing during the course of the experiment. In other words, as the eggs aged, the cortex became progressively less rigid. However, this was not a serious difficulty for we made measurements of the control eggs from time to time, so that the value finally recorded was the appropriate value for comparison with that of the experimental eggs.

The results of our studies are shown in Table I. This table does not give the time at which the measurements were made. In almost all cases the effect of the

[2] These hand centrifuges can no longer be purchased from the Emerson Co., but they can be supplied at a reasonable price by Mr. J. A. Appenzeller of the Zoology Department of the University of Pennsylvania.

environment on the cortex was rapid, so that probably time did not play much of a role in determining the magnitude or the nature of the effect. However, calcium chloride seems to be rather slow in its action, so that a pronounced effect of the calcium apparently does not occur until about half an hour or longer.

TABLE I

The effect of various agents on the rigidity of the protoplasmic cortex

Treatment	Centrifugal force in gravities	
	Experimental	Control
Potassium oxalate (70 pts. s.w. + 30 pts. 0.35 *M* K oxalate)	1600	4000
Potassium chloride (0.53 *M*)	1700	4400
Calcium chloride (0.3 *M*)	8400	6400
Sodium hydroxide (pH 9.34)	1200	3200
Ammonium hydroxide (pH 9.65)	600	5100
Hydrochloric acid (pH 3.78)	3900	6100
Ether (2%)	1400	5100
Butyl alcohol (1.5%)	1300	5600
Amyl alcohol (0.5%)	1600	6000
Heat (38° C.)	3200	5400
Cold (0° C.)	3000	4900
Ultraviolet	1100	4000

The table does not give information as to how the eggs were exposed and further detail is necessary. When eggs were placed in solutions containing potassium oxalate, they were washed at least once in the oxalate solution, so as to avoid the presence of too many oxalate crystals. Eggs exposed to potassium chloride solutions were washed several times in the solution; this was to get rid of traces of calcium. In studying the effect of sodium hydroxide, normal NaOH was added to sea water. Solutions of various pH's were tested; pronounced effects were obtained with a solution having a pH of 9.34. Likewise, in studying the effect of ammonium hydroxide, solutions of varying pH were used. We are not prepared to state exactly how much alkalinity is necessary to produce a marked change in the cortex. With ammonium hydroxide, a pH of 8.9 does produce liquefaction, although this is not as pronounced as that which occurs in more alkaline solutions. In experiments with fat solvents we used as high a concentration as we could without destroying the eggs. Thus, for example, in our experiments with butyl alcohol, eggs treated with a 2% solution (by volume) broke up when centrifuged. A 1% solution had little effect on the eggs; a 1.5% solution had an effect without injuring the eggs too greatly. It should be noted that the concentrations of all fat solvents listed in the table are expressed as volume per cent.

In the heat experiments, a temperature of 36° C. had about as much effect as did a temperature of 38° C. In our experiments with cold, we found that moderately cold temperatures were without effect; only when eggs were exposed to temperatures near 0° C. was the cortex markedly affected. In our experiments with ultraviolet, we used a powerful Uviarc lamp. This lamp gives off a whole spectrum of radiations, but we were concerned not so much with determining which wave lengths were effective or how much energy was involved, but rather with understanding what happened to the cortex when the eggs were stimulated by ultraviolet. However, we were careful to exclude the effect of heat. Accordingly, eggs to be irradiated were exposed in Stender dishes immersed in fingerbowls contain-

ing cold sea water (at about 10° C.). Under these conditions the sea water in the Stender dishes containing the eggs did not warm up above the room temperature. Eggs were exposed for 4 minutes; the target distance was 9.5 centimeters.

Let us now attempt to answer the first of the two questions we proposed for consideration. Are the properties of the gel in the cortex of the Chaetopterus egg similar to those of the gel in the cortex of the ameba? In ameba, potassium ion markedly decreases the viscosity of the cortical gel and calcium increases it (Heilbrunn and Daugherty, 1932). The gel in the cortex of the ameba tends to be liquefied by oxalate (Heilbrunn and Daugherty, 1933); the indication is that the Chaetopterus cortical gel acts in the same way, although the effect produced by potassium oxalate may have been due to potassium ion. Ether, butyl alcohol and amyl alcohol all tend to cause liquefaction of the Chaetopterus egg, just as they do in ameba (Daugherty, 1937). Cold and heat likewise exert a liquefying effect in both the cortex of Chaetopterus and that of ameba; ultraviolet likewise has a similar effect on both. There are no published data on the effect of alkali and acid on the cortex of ameba, but various workers in the Zoological Laboratory of the University of Pennsylvania have shown in unpublished experiments that alkaline solutions cause a very marked decrease in the viscosity of the ameba cortex just as they cause a drop in the rigidity of the Chaetopterus cortical gel. Indeed, there appears to be only one point of difference between the behavior of the Chaetopterus cortex and the behavior of the cortex in ameba. Acid solutions cause a decrease in the rigidity of the Chaetopterus cortex. On the other hand, unpublished experiments indicate that acids have the opposite sort of an effect on the ameba cortex. On the whole, however, there is a marked similarity in the behavior of the two types of gels. Moreover, Berwick (1951) has shown that a fat solvent like ether causes a release of calcium from muscle brei, and Weimar (unpublished experiments) has found that cold, heat, ultraviolet and potassium all have a similar effect on brei from homogenized frog muscles.

Now as to the second of the questions we proposed. Stimulating agents (heat, cold, electric shocks, ultraviolet radiation) all cause a liquefaction of the cortex of ameba. And as is apparent from Table I they likewise all cause a sharp drop in the rigidity of the cortical gel in the Chaetopterus egg. Moreover, all the agents which stimulate the Chaetopterus egg to divide and begin its development cause a liquefaction of the cortex. Many years ago, Loeb (1901) showed that potassium ions cause an initiation of development in the Chaetopterus egg. We repeated these experiments with isotonic potassium chloride and again found that cleavage was initiated. In one experiment, a 14 minute exposure to a 0.53 molar potassium chloride solution gave 21% cleavage, and a 27 minute exposure gave 34% cleavage. Some of these cleavages look rather normal, but some might not have been true cleavages; it would not have been possible to decide without making cytological preparations.

Alkalies also can cause the initiation of cleavage and development in Chaetopterus. Something of this sort is mentioned by Loeb in his paper, but he is not sure whether the effect is due to alkali or to potassium ions. In one of our experiments in which eggs were kept in a solution of sea water to which sodium hydroxide had been added until the pH was 9.34, 59% cleaved. These eggs had been subjected to the alkali for nearly 5 hours (292 minutes). In another experiment, there was a high percentage of cleavage in eggs left in solutions made alkaline with sodium

hydroxide. The pH of these solutions was 8.67 and 8.96. Of all the parthenogenetic agents used by Loeb, the most successful were hypertonic solutions. We therefore were interested in trying to find out whether such solutions caused a liquefaction of the cortex. The evidence that we have indicates that they do. But the tests are complicated by two factors. In the first place, if strong hypertonic solutions are used, the interior protoplasm of the cell becomes so stiff that even vigorous centrifuging does not move the granules in the interior of the cell. Under these conditions the granules of the cortex can not properly be observed, for it is only when there is a hyaline zone free from granules that the cortex stands out as a distinct layer which can readily be studied. In the second place, hypertonic solutions cause a pronounced swelling of the cortical granules.

In our tests when 10 ml. of 2.5 molar sodium chloride was added to 90 ml. of sea water, no great amount of cleavage occurred. However, if 15 ml. of the hypertonic solution was added to 85 ml. sea water or when 20 ml. of the hypertonic solution was added to 80 ml. of sea water, high percentages of parthenogenetic development were obtained. In one experiment eggs were exposed to 15 ml. of 2.5 molar sodium chloride plus 85 ml. sea water for 13, 24, 49, 69 minutes, and then returned to sea water. These exposures gave 34%, 38%, 57%, and 58% cleavage, respectively. In this experiment a centrifuge test was made one minute after the cells were placed in the hypertonic solution. At this time, the cortex of the eggs exposed to the hypertonic solution was decidedly less rigid than the cortex of the control eggs in sea water. However, because of the factors mentioned above, no quantitative measure was possible.

Heat and cold can also cause the egg of the Chaetopterus to cleave. No great attempt was made to discover exactly which exposures gave the best results. In one experiment in which eggs were heated to 36° C. and then allowed to cool slowly, 34% of the eggs cleaved. Exposures to cold did not give such high percentages. In one experiment an exposure to 0° C. for two minutes gave 11% cleavage; in another experiment, eggs exposed to − 0.5° C. for two minutes gave 9% cleavage.

One thing is certain: all the agents which have been shown to cause initiation of cell division and initiation of development in the Chaetopterus egg cause a decrease in the rigidity of the cortex. But not all the agents which cause this change in the cortex are effective in initiating cleavage. This is due to the fact that some of the agents we tried are very toxic, and the eggs are killed by them before there is any chance for development.

The cortex of the Arbacia egg is much more difficult to study than is the cortex of the Chaetopterus egg. The cortical granules in the Arbacia egg are decidedly smaller than the cortical granules in the Chaetopterus egg. This makes them harder to see and it also makes them harder to move by centrifugal force, for according to Stokes' law the force required to move a particle varies as the square of the radius of the particle and thus the force required would be much greater for a small than for a large particle. We have made only a preliminary study of the cortex of the Arbacia egg, but our study has gone far enough to show that it has the same type of structure as the cortex of the Chaetopterus egg. In the Arbacia egg also, the cortex tends to be liquefied by oxalate and by ether. Moreover, just as in the Chaetopterus egg, the Arbacia cortex tends to become less rigid as the egg ages.

Both in the Chaetopterus egg and under some conditions in the Arbacia egg, it is possible to move the cortical granules to one side of the cell. This makes possible a study of the influence these granules may have on the organization of the egg. In earlier centrifuge studies on egg organization it was possible to move only the granules in the egg interior. If, after suitable liquefying treatments, the granules of the cortex are moved also, this may have an important effect on the future development of the egg.

Summary

1. The cortex of the Chaetopterus egg is made more rigid by excess calcium; it tends to lose its rigidity when exposed to oxalate solutions or to potassium ions.

2. The cortex of the Chaetopterus egg is also liquefied by acid, alkali, fat solvents, heat, cold, ultraviolet radiation and hypertonic solutions.

3. In general, the behavior of the Chaetopterus egg cortex is very like that of the cortex of ameba.

4. All agents which have been found to stimulate the Chaetopterus egg to divide cause a liquefaction of the cortex.

5. The results of this investigation lend additional support to the colloid chemical theory of stimulation.

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